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CLINICAL AND EXPERIMENTAL

DOES REMOVAL OF A NORMAL GALLBLADDER AFFECT THE METABOLISM OF LIPIDS?*

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TWO general functions are generally assigned to the gallbladder, namely, the regulation of pressure of the biliary passages, and storage of bile for digestive purposes. Certain experimental observations have been reported by Sweet[1] which indicate that the gallbladder may be related to lipid metabolism. His data are sufficiently striking to warrant a repetition and extension of his experiment.

Sweet fed a normal dog a fat meal and determined the cholesterol content of the blood at three hour intervals for twelve hours. Having determined the normal cholesterol curves, he removed the gallbladder. He found that after cholecystectomy the blood cholesterol following a fat meal rose to almost double the normal values. About forty days after operation the blood cholesterol response returned to normal, and was less than normal at seventy-four days. In one observation three hundred days after operation, the cholesterol curve approached normal. He also noted a change in the contour of the cholesterol curve following fat ingestion. Normally the peak of the rise occurred at the sixth hour, whereas after cholecystectomy the peak was reached at the ninth hour. The increase in blood cholesterol was reported to be due chiefly to a rise in cholesterol esters.

Fowweather and Collinson[2] found the blood cholesterol values in man normal or subnormal several months after cholecystectomy. This is not necessarily contrary to Sweet's findings in dogs.

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We thought it possible that a more continuous flow of bile into the intestine after removal of the gallbladder might modify the absorption rate of fat. However, not only the rate of absorption of lipids into the blood might be a factor in interpreting Sweet's results, but other factors such as tissue injury might modify the alimentary lipemia curve. With these possibilities in mind, the experiment of Sweet has been repeated and extended.

METHODS

Normal absorption curves in dogs for fat similar to the dextrose tolerance test were determined. The dogs were fasted for three days because Rony and Ching[3] obtained more uniform alimentary blood fat curves after three days of

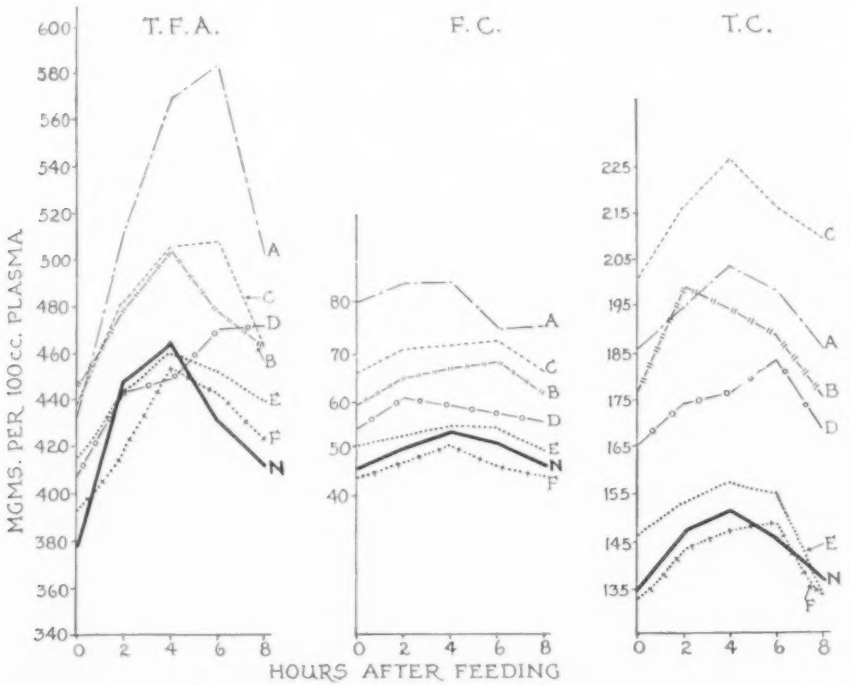

Fig. 1.—A composite curve of nine dogs showing the effect of cholecystectomy on blood lipids. T.F.A., total fatty acids; F.C., free cholesterol; T.C., total cholesterol. *N*, control curve; *A*, six days after operation; *B*, twenty-four days; *C*, forty-two days; *D*, sixty-seven days; *E*, six months; *F*, one year.

fasting. Then the blood plasma was analyzed for free cholesterol by M. Yasuda's[4] method, for total cholesterol by R. Okey's[5] method, and for total fatty acids by W. R. Bloor's[6] process. The dogs were then given by stomach tube 2 c.c. of olive oil per pound body weight, and four more blood specimens were taken at two-hour periods for analysis. After obtaining several normal blood lipid curves the gallbladder was removed and the experiments were repeated at intervals for several months.

Mange had to be avoided because it was found that three days of fasting in the presence of mange augmented the fasting level of plasma lipids. The same will probably occur if the dog is not given good dietary and hygienic care.

RESULTS

Repetition of Sweet's Experiment.—Nine dogs were studied according to the experimental outline just given. Composite curves of the results are shown in Fig. 1. These curves are not unlike those obtained by Sweet, and constitute essentially a confirmation of his observations.

It was found that in the dogs operated upon there was an increase in the third or sixth day fasting level of blood lipids. However, the blood lipid curves

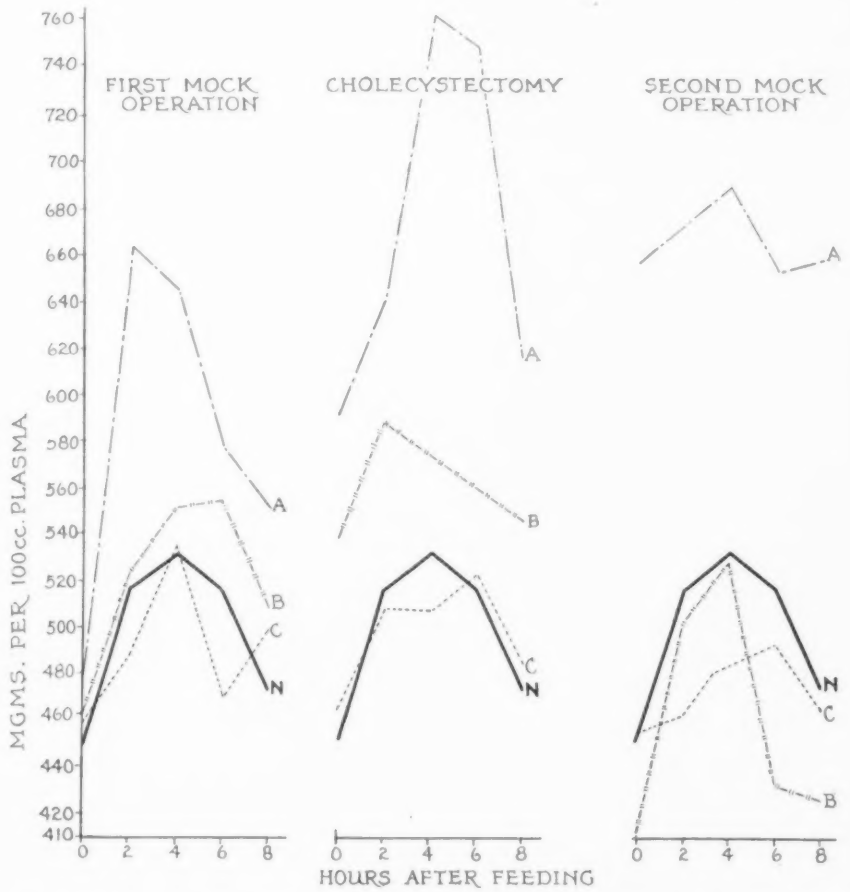

Fig. 2.—A composite curve of four dogs showing the effect of a mock operation, cholecystectomy and a second mock operation on total fatty acids. *N*, normal control; *A*, three days postoperatively; *B*, twenty days; *C*, thirty-two days after mock operation, eighty-five days after cholecystectomy and second mock operation.

after fat ingestion were not absolutely uniform either before or after cholecystectomy, and the peak of the rise did not occur at a definite hour. The fasting levels occasionally returned to normal within a month after operation. In one dog the third day fasting level of total fatty acid and total cholesterol remained high for one year after cholecystectomy. This dog for some unknown reason remained in poor condition after the operation. We consider the "A," "B," and "C" curves to be significant elevations of the lipid values.

A Control Group of Experiments.—"Mock operation": It was then thought advisable to determine whether the results obtained were specifically due to removal of the gall bladder, or whether they could be produced by stripping out a piece of the hepatic capsule and manipulating, without removing, the gallbladder. Such a control was considered to be important because of the known relation of liver injury to blood plasma cholesterol values.

The results on four dogs in general were similar to those obtained by cholecystectomy. The "mock operation" was followed by a rise in the level of blood lipids after three days of fasting. The response to fat feeding also was modified as if the gallbladder had been removed.

Of course, the manipulation of the gall bladder may have injured it. So, experiments were performed on four dogs on which a "mock operation" was

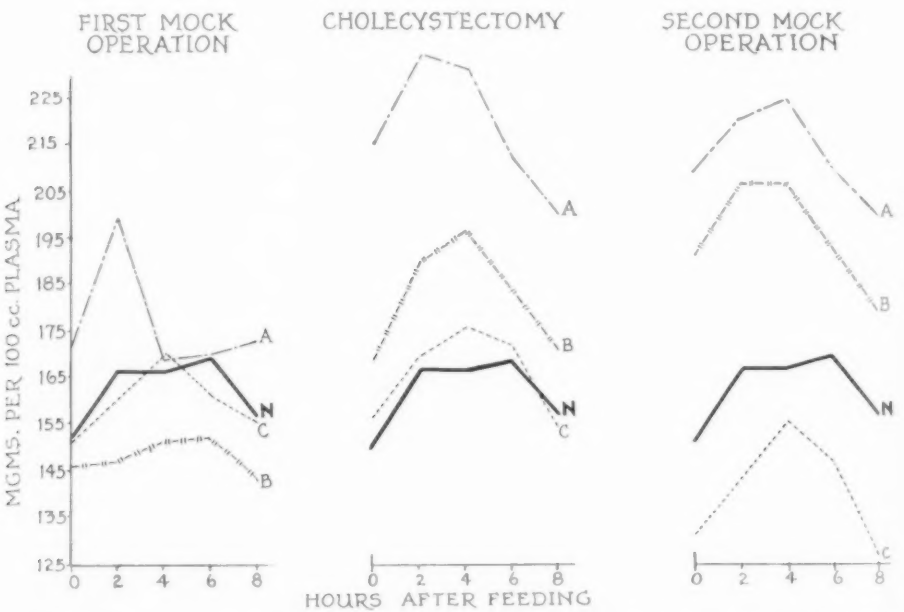

Fig. 3.—A composite curve of four dogs showing the effect of a "mock operation," cholecystectomy, and a second mock operation on total plasma cholesterol. *N*, normal control; *A*, three days postoperatively; *B*, twenty days; *C*, thirty-two days after mock operation, eighty-five days after cholecystectomy and second mock operation.

performed without manipulating the gallbladder. However, after the plasma fat changes had returned to normal, the gallbladder was removed, and then after the plasma fat changes had returned to normal another "mock operation" was performed.

The essential results are shown in the composite curves in Figs. 2, 3, and 4. More frequent tests were performed than those indicated in the figures, but it is unnecessary to take space by the presentation of all the data. The curves, however, are an accurate representation of the average trends in the various animals. It is to be noted that the mock operations influenced the results less than the cholecystectomy. Also, the effect of cholecystectomy tended to be longer in duration.

Another dog was fed a quantitative diet every day. The twenty-four-hour postcibal blood fat levels were determined successively before and after ether anesthesia, trauma to the liver but not the gallbladder, and subsequent cholecystectomy. At times this dog showed some increase in the twenty-four-hour postcibal blood lipid values, but on the average the levels were but slightly higher than is the normal variation for the same dog (Table I). This dog showed a poor response to cholecystectomy.

TABLE I

THE RESULTS OF CHOLECYSTECTOMY AND OTHER PROCEDURES ON BLOOD FAT LEVELS TWENTY-FOUR HOURS POSTCIBUM

DATE	TOTAL FATTY ACID MG./ 100 C.C.	FREE CHOLESTEROL MG./ 100 C.C.	TOTAL CHOLESTEROL MG./ 100 C.C.	AVERAGE TOTAL FATTY ACID	AVERAGE FREE CHOLESTEROL	AVERAGE TOTAL CHOLESTEROL	AVERAGE CHOLESTEROL ESTER
4/20/35	365	33.4	110				
4/22/35	377	32.4	107				
4/24/35	508	53.3	165				
4/26/35	393	41.8	115				
4/29/35	354	40.8	118				
5/ 1/35	408	40.8	113	399.7	40.9	120.8	80
5/ 3/35	393	43.9	118				
5/ 5/35				Dog fasted 48 hours			
5/ 6/35	362	41.8	131				
5/14/35				Deep ether anesthesia for one hour			
5/15/35	470	44.9	152				
5/17/35	524	31.4	115				
5/20/35	393	33.4	94				
5/22/35	300	34.5	105				
5/24/35	339	32.4	110				
5/27/35	462	39.7	84	421.8	37.9	112	74
5/29/35	424	34.5	115				
5/31/35	454	42.8	107				
6/ 3/35	431	48.0	128				
6/ 6/35				Laparotomy, manipulated gallbladder (ether anesthesia)			
6/ 7/35	501	46.0	121				
6/10/35	485	51.2	131				
6/12/35	501	44.9	115				
6/14/35	323	43.9	173	445	46.8	146	99
6/17/35	416	48.1	191				
6/18/35				Removed gallbladder (ether anesthesia)			
6/19/35	447	52.3	173				
6/21/35	454	70.0	170				
6/24/35	554	69.0	173				
6/26/35	431	50.2	131				
6/28/35	447	42.8	155				
7/ 1/35	385	39.7	89	440.03	50.7	131	80
7/ 3/35	408	40.8	113				
7/ 5/35	470	47.0	92				
7/ 8/35	416	50.2	113				
7/10/35	470	41.8	94				
7/12/35	362	54.2	141				

Thirty-pound male dog. Quantitative diet: 300 c.c. milk, 10 c.c. cod liver oil, 12 gm. yeast, 200 gm. bone, 200 gm. Pard (Swift & Co. analysis: Protein 10.5 per cent, carbohydrate [N.F.E.] 10 per cent, fat 2.50 per cent, crude fiber [max.] 50 per cent).

Two other dogs were fed the quantitative diet, but were fasted for three days before determining blood fat levels. When this had been repeated several times, a laparotomy was performed, and the fasting and alimentary lipemia blood lipid levels were elevated.

When the levels again approached the normal, a cholecystectomy was performed. Again the blood lipid levels were elevated. When the levels had returned to normal after the second operation, another laparotomy was performed. The ensuing fasting blood lipid levels were elevated. Fig. 5 shows the composite curves of two dogs before operation, after laparotomy, after cholecystectomy, and after a subsequent laparotomy. It should be remembered that these

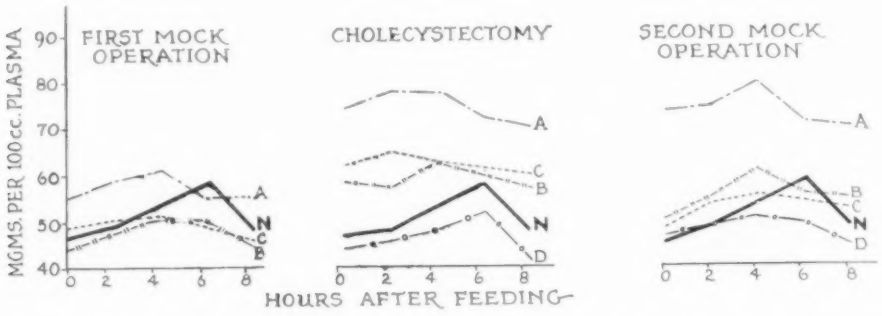

Fig. 4.—A composite curve of four dogs showing the effect of mock operation, cholecystectomy and a second mock operation on free plasma cholesterol. The lettering of the curves means the same as indicated for Figs. 2 and 3.

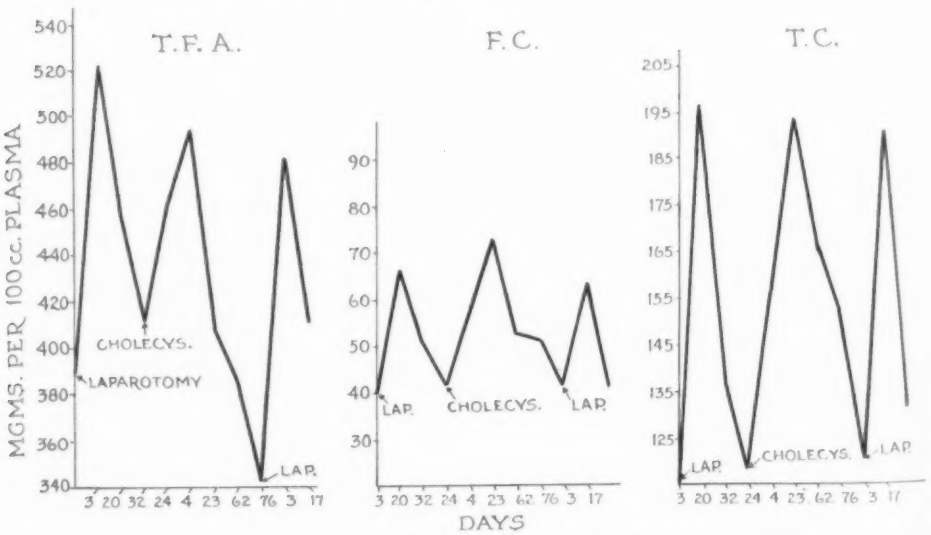

Fig. 5.—Showing the changes in total fatty acids (*T.F.A.*), free cholesterol (*F.C.*) and total cholesterol (*T.C.*) after a laparotomy with liver trauma, cholecystectomy, and a second mock operation.

dogs were fasted for three days before each study of the blood lipid levels. It is to be noted that in this group the laparotomy was as effective in causing changes as the cholecystectomy.

Although the twenty-four-hour postcibal blood fat levels were unchanged after ether anesthesia, the effect of a three-day fast after anesthesia was unknown. Four dogs were fed a quantitative diet and then the blood lipid levels were determined after fasting for three days. In two of the dogs the alimentary lipemia response to olive oil was also studied. After establishing the

normal averages for the blood fats under these conditions each dog was anesthetized with nembutal, ether and chloroform in turn. Nembutal anesthesia did not change the third day fasting lipid levels nor modify the lipemia curve in response to olive oil ingestion. Ether and chloroform anesthesias both raised the third day fasting levels and the alimentary lipemia curves. Chloroform had a more marked and prolonged effect than ether, although the effects were not evident one or two weeks later.

DISCUSSION

It is not surprising that slight injury to the liver changes the blood lipid response to starvation or to ingested fat. In some of the dogs, the effect of cholecystectomy was of longer duration than that of liver trauma. Cholecystectomy is accompanied by slight liver damage, which occurs late, since Bergh, Sandblom, and Ivy[5] have shown that following removal of a normal gallbladder in a dog small areas of necrosis, undetectable by means of clinical tests, are found in the liver.

This work was undertaken with the hope that some general function of the gallbladder as related to lipid metabolism might be discovered. The results do not indicate that the gallbladder possesses such a general function. Any change that occurred following cholecystectomy can be attributed clearly to the resulting liver injury.

Since cholesterol is eliminated by the intestinal mucosa to a very significant extent, it would be of interest to determine the effect of cholecystectomy on the elimination of cholesterol and fat in the feces. Until this is done one cannot say that the reservoir function of the gallbladder plays no rôle in the metabolism of lipids.

CONCLUSION

The rise in fasting plasma lipid levels obtained after cholecystectomy is not specifically due to removal of the gallbladder; it may also be obtained after liver damage, tissue injury, and for a short time after ether or chloroform anesthesia. By studying the blood lipids (total fatty acids, free and total cholesterol) before and after cholecystectomy both under the condition of fasting and during the digestion and absorption of fat, it could not be demonstrated that the gallbladder possessed a function of regulating the metabolism of lipids.

We would like to express appreciation to Miss Vivian Bass for technical assistance in this work.

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THE PRODUCTION OF PHOSPHATE RICKETS IN RATS IN THE PRESENCE OF VITAMIN D*

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A CRITICAL review of the literature gives one the impression that phosphate rickets can not be produced in rats in the presence of adequate vitamin D. A diet of much higher calcium to phosphorus ratio than has previously been used was planned. The following diet is very low in phosphorus and protein, adequate in calcium, and fulfills the remainder of the nutritional requirements of rats.

TABLE 1

FOOD	PER CENT IN RATION	PHOSPHORUS IN GM. IN 100 GM. OF RATION	CALCIUM IN GM. IN 100 GM. OF RATION
Corn	76	0.144	0.014
Oats	20	0.079	0.014
Calcium carbonate	3	----	1.200
Sodium chloride	1	----	----
Total	100	0.223	1.228

Ten milligrams of ferric citrate and 1 c.c. of cod liver oil containing 250 vitamin D units were added to the above ration which was fed each rat every other day. An excess of food was kept in the cages at all times. A control group was fed the following diet which is higher in phosphorus and protein. The ferric citrate and cod liver oil were added as in the low phosphorus diet. The meat powder was prepared from beef round from which the visible fat had been removed by cutting. The lean meat was ground, desiccated in vacuum at 56° C., and pulverized.

Ten disease-free white female rats from the Wistar strain and twenty-eight days old were fed the low phosphorus diet for one hundred and thirty-two days. An identical group was fed the diet higher in phosphorus. At the end of the feeding period the long bones were x-rayed, the gastrointestinal tract removed,

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TABLE II

FOOD	PER CENT IN RATION	PHOSPHORUS IN GM. IN 100 GM. OF RATION	CALCIUM IN GM. IN 100 GM. OF RATION
Corn	5	0.009	0.001
Oats	50	0.198	0.034
Meat powder	41	0.227	----
Calcium carbonate	3	----	1.200
Sodium chloride	1	----	----
Total	100	0.434	1.235

and the animals incinerated in an electric furnace. The ash was analyzed for calcium by the volumetric oxalate method and for phosphorus by the amino naphthol sulphonic method.

RESULTS

The average of the percentage of calcium in the ash of the animals fed the low phosphorus diet was 32 as compared to 40 in those fed the diet higher in phosphorus. The average of the percentage of phosphorus in animals fed the low phosphorus diet was 12 as compared to 6 found in those fed the higher phosphorus diet.

TABLE III

ANALYSIS OF RATS FED ON LOW PHOSPHORUS DIET

RAT	WEIGHT AT END OF EXPERIMENT IN GM.	WEIGHT OF ASH IN GM.	WEIGHT OF CALCIUM IN GM.	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM.	PER CENT OF PHOSPHORUS IN ASH
A	36	1.530	0.490	32	0.200	13
B	32	1.550	0.496	32	0.195	13
C	36	1.521	0.426	28	0.211	14
D	36	1.460	0.467	32	0.205	14
E	48	1.930	0.618	32	0.190	10
F	56	1.881	0.658	35	0.200	11
G	36	1.491	0.477	32	0.189	13
H	32	1.611	0.516	32	0.190	12
I	36	1.650	0.561	33	0.205	12
J	30	1.550	0.527	34	0.202	13
Average				32		12

TABLE IV

ANALYSIS OF RATS FED ON HIGH PHOSPHORUS DIET

RAT	WEIGHT AT END OF EXPERIMENT IN GM.	WEIGHT OF ASH IN GM.	WEIGHT OF CALCIUM IN GM.	PER CENT OF CALCIUM IN ASH	WEIGHT OF PHOSPHORUS IN GM.	PER CENT OF PHOSPHORUS IN ASH
AA	160	5.980	2.332	39	0.426	7
BB	168	5.912	2.306	39	0.421	7
CC	212	6.981	2.722	39	0.421	6
DD	172	6.660	2.600	39	0.480	7
EE	188	6.256	2.503	40	0.387	6
FF	200	8.351	3.507	42	0.414	5
GG	220	7.696	3.309	43	0.400	5
HH	212	7.760	2.949	38	0.463	6
II	172	6.532	2.548	39	0.400	6
JJ	180	6.331	2.469	39	0.392	6
Average				40		6

The blood counts and hemoglobin determinations by the Sahli method on all of the animals were normal at the end of the feeding period.

X-ray pictures taken of the long bones of the animals that were fed the low phosphorus diet were typical of rachitic bones. The bones bowed on pressure, the epiphyseal line was jagged and irregular, and there was a marked diminution in the calcification at the epiphysis. X-ray pictures taken of the long bones of animals fed high phosphorus diets were normal.

CONCLUSIONS

A diet low in phosphorus and protein, adequate in calcium and vitamin D, but with a calcium to phosphorus ratio of 5.5 to 1 will produce rickets when fed to rats. When the ratio is lowered to 3 to 1 by raising the phosphorus content by adding meat, the bones develop normally.

It will be noted that the percentage of phosphorus in the bones of the rachitic rat is higher and the calcium lower than that in the bones of the normal animal. This difference in composition is probably due to a higher percentage of the calcium present as the carbonate in the normal than in the rachitic bones.

V. NONRENAL AZOTEMIA*

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SINCE our report[1] in 1935, of various clinical conditions in which high blood urea values arise and in which there is no evidence of chronic glomerulonephritis, a number of other conditions have come under our observation. A number of important papers have also appeared which bear directly or indirectly on the problem of hyperazotemia of nonrenal origin.

In the discussion of our earlier presentation, we made an analysis of the various states with which high blood urea is associated and found that there was one large group in which the majority of clinical cases could be placed and concerning which there was a considerable amount of experimental data. This type which others had designated hypochloremic uremia or "Azotemie par manque de sel" appears to be closely connected with a severe, but reversible change in the salt and water metabolism of the organism, and proved to be the type which responds favorably to salt and water administration. Recently McCance[2] has produced a similar clinical condition by salt deprivation alone through salt-free diet combined with sweating.

There are other states of nonrenal azotemia that are not conditioned by demonstrable loss of water or electrolytes. There is a possibility that such a state may reflect some internal metabolic derangement which at present is not

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readily discernible. Thus, the injection of histamine into animals produces an increase in nitrogen in the plasma with a drop in the chlorides. There is no evidence of external loss of water and electrolytes through known channels, such as the stomach, the intestines, or the skin.[3]

Indeed, we called attention to the many points of similarity, both chemical and clinical, between the hypochloremic states and the crises of Addison's disease.[1] In more recent experimental work, Wohl, Burns, and Clark found morphologic alterations in adrenal glands of dogs, with hypochloremia resulting from experimental high intestinal obstruction.

With replacement of large quantities of normal salt solution (400 c.c. daily) without cortin or with smaller amounts of normal salt solution (200 c.c. daily) plus cortin, the clinical and biochemical signs of the toxemia abated,

TABLE I

CLINICAL CONDITIONS OF NONRENAL AZOTEMIA

Dehydration from:

Loss of gastrointestinal secretions:

a. Cancer of stomach
b. Pylorospasm
c. Hyperemesis gravidarum
d. Gastric tetany
e. Alkalosis in alkaline treatment of peptic ulcer
f. Acute intestinal obstruction
g. Self-treatment with duodenal tube
h. Diarrhea

Loss of extracellular fluid:

a. Profuse sweating from exposure to extreme heat
b. Diabetes mellitus
c. Postoperative
d. Therapeutic purposes: limitation of fluid (epilepsy)

Nonobvious loss of fluids and electrolytes:

a. Burns
b. Histamine shock and other shocklike conditions
c. Adrenal insufficiency

and the adrenal glands remained intact[4, 5] (Fig. 1) (see Charts 1, 2). The various symptoms of cortico-adrenal insufficiency were explained by Zwemer and Truszkowski in terms "of a disturbance of cortico-adrenal potassium interrelations."[6] Scudder, Zwemer, and Truszkowski fortified our contention that acute intestinal obstruction and adrenal insufficiency have many features in common, by demonstrating high blood potassium values in acute intestinal obstruction.[7] The report of Harrop and his associates[8] on the salt-water hormone of the adrenals is provocative of the thought that a functional and temporary derangement of the adrenals may be associated fundamentally with some states of nonnephritic urea increases under discussion; the latter eventuating from fluid and base alterations between the extracellular tissues, the tissue cells, and the blood. The appearance of a high figure of blood urea nitrogen in any clinical case is properly regarded as pointing first to renal damage, especially if to this is added the presence of albumin and casts, as well as the manifestations of weakness or vertigo, confusion, or stupor. With our present knowledge, however, the possibility of other conditions causing this state of affairs ought to

make the physician complete the picture of Bright's disease before he consigns his patient to that sort of prognostic limbo. The blood pressure, the eye-ground pattern, the specific gravity of the urine, the kidney function tests, and the history of a previous illness leading to kidney disease, when kept in mind, will aid in differentiating true uremia from nonrenal hyperazotemia. The correct and early diagnosis may mean not only rapid clearing of toxic symptoms by intravenous saline administration, but may prevent the patient from going into deep coma and possibly death. We stressed in our first report, dehydration as the common denominator of the azotemia and hypochloremia and felt that some of the cases with obvious loss of secretions from the gastrointestinal tract,

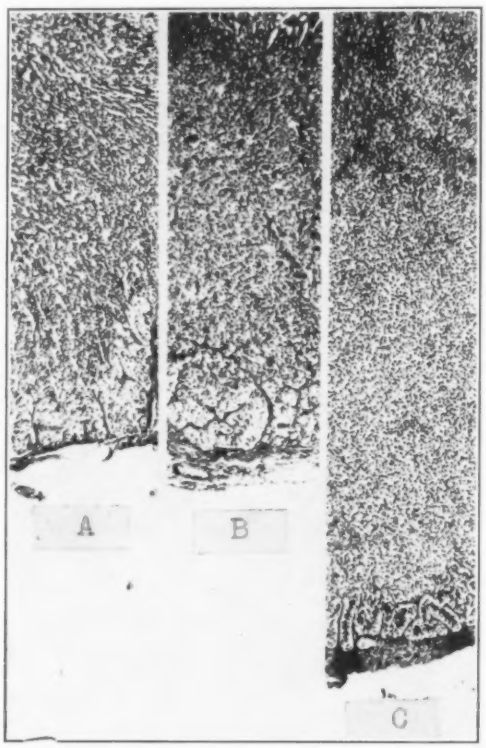

Fig. 1.—Low power photomicrograph of adrenal cortex in experimental high intestinal obstruction (all photomicrographs made at the same magnitude). *A*, extreme lipoid exhaustion; zona glomerulosa "moth eaten" with rupture of cell walls; zona fasciculata: cytoplasm granular, apparently devoid of lipoid; *B*, partial lipoid depletion (dog receiving insufficient quantities of salt solution); zona glomerulosa filled with lipoid; cell walls preserved; zona fasciculata only partially depleted; cytoplasm contains lipoid droplets; *C*, no evidence of lipoid depletion; "storage phase" (dog receiving sufficient quantities of salt solution); zona glomerulosa and zona fasciculata well filled with lipoid; cells foamy; note increased width of cortex as compared with *A* and *B*.

forced limitation of fluid and salt intake for therapeutic purposes (epilepsy), or postoperatively belong to this group; but we had no case like the following, the pathogenesis of which remains obscure.

CASE 1.—A colored boy, ten years old, was admitted to the Philadelphia General Hospital on March 14, 1936, with a history of weakness, "inward fever" cough, abdominal pain, anorexia, and constipation. He had been in good health up to Feb. 29, 1936, when there was gradual and progressively increasing weakness and loss of energy. He developed "some

fever," with a cough afterward. Anorexia and constipation were profound from the beginning, but there had been no vomiting until five days after he was forced to bed because of weakness. Examination disclosed an emaciated, weak, and apathetic colored boy, tossing about in bed. There was no vomiting, except once when milk feeding was attempted. The blood pressure was 80 systolic; diastolic was unobtainable. Lungs were clear; heart sounds distant and slow; and the abdomen was scaphoid. There were no masses or tenderness. The spleen was not palpable. The temperature range was normal.

A diagnosis of marasmus, dehydration, and gastrointestinal upset was made. The laboratory studies were as follows: Urine: acid, specific gravity 1.019. Albumin and sugar negative. No acetone; no casts were found; leucocytes and erythrocytes, occasional. Blood count: erythrocytes 4,460,000; leucocytes 26,000; hemoglobin 13.6 gm.; polymorphonuclears 82 per cent; lymphocytes 18 per cent. Blood: Kahn negative; blood sugar 160.0; urea nitrogen 260 mg. per cent. On March 16, 1936: blood sugar 121.0 and urea nitrogen 170 mg. per cent. On March 17, 1936: creatinine 5.0 Blood chlorides 694 mg. per cent; blood CO_2, 41 volumes per cent. The boy was placed on continuous hypodermoclysis of 3 per cent glucose in normal salt solution and began to improve at once. In four days his blood pressure was 102/60, but he had a bradycardia of 58 with some extrasystoles. On March 23, 1936, the blood urea nitrogen was 20 mg. per cent; the blood chlorides were 610 mg. per cent and the patient

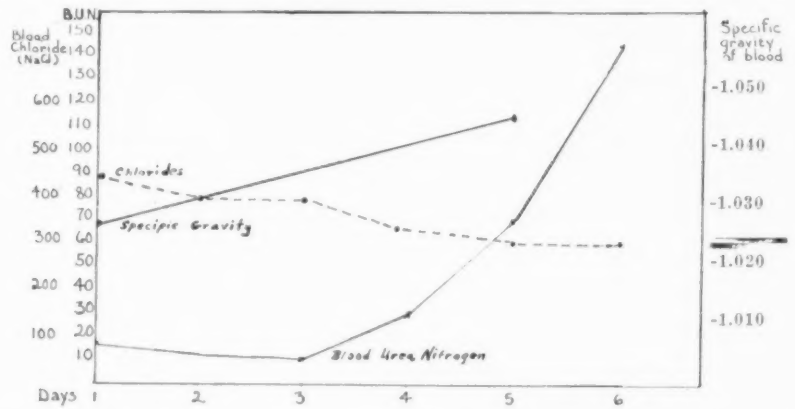

Chart 1.—Blood findings in experimental high intestinal obstruction showing marked rise in urea nitrogen and specific gravity of blood; decrease in blood chlorides. (Wohl, Burns, and Pfeiffer.[3])

was entirely better, happy, and eating heartily. The spinal fluid was negative, except for a chloride content of 956 mg. per cent during salt administration. The urea clearance on March 23, 1936, was 59 per cent the first hour, 72 per cent the second hour.

This boy returned for examination on May 22, 1936, after a healthy interim and his urinalysis was perfectly normal (seven specimens were examined). The blood urea nitrogen ranged from 21 to 10 mg. per cent. The urea clearance was 165 per cent, first hour clearance, 131 per cent second hour. The phenol sulphonephthalein was 73 per cent in one hour. The spinal fluid was negative. Physical examination, x-ray studies of chest and gastrointestinal tract furnished no clue as to his former illness.

Comments.—The prolonged malnutrition with its accompanying decrease in the blood volume would appear to be the probable underlying basis for the increase in blood urea. There must have been an undue loss of intracellular fluid with an acidosis. The CO_2 was 41 volumes per cent. It is worthy to note that at the time the blood urea had arisen, the blood chlorides remained at a normal level. This would strengthen the point of view that the hyperazotemia may occur without the hypochloremia. This is contrary to Blum's contention that hypochloremia is primary and the azotemia compensates for the

low osmotic pressure of body fluid resulting from loss of electrolytes. Furthermore, from a practical therapeutic consideration, it should be stressed that not all the patients with hyperazotemia should be given salt solution. In patients who show normal blood chlorides, dextrose in distilled water is preferable. This procedure may avert subsequent edema and irritative cerebral phenomena (hydration of the brain) if excessive salt solution is given in cases with normal blood chlorides. On the other hand, this boy's condition might conceivably have been an acute but temporary functional derangement of the adrenals, with alteration of the intra- and extracellular fluid and electrolyte distribution. Whether an abnormal release of potassium might account for the symptoms of low blood pressure, asthenia, and anorexia cannot be definitely stated. Unfortunately, potassium studies were not done in this case.

It is not generally appreciated that increased blood urea nitrogen figures occur in gastrointestinal hemorrhages, although, recently, it has been commented upon in the literature.[9, 10, 15, 16] Of course, it has, for a long time, been known that experimentally sudden loss of blood in itself will be followed by an

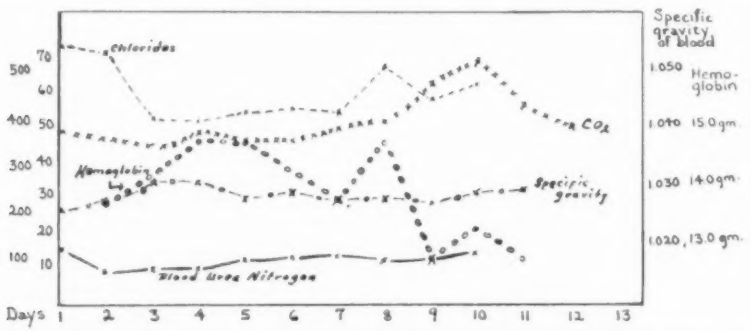

Chart 2.—Experimental high intestinal obstruction treated with extract of adrenal cortex and salt solution. Note the insignificant change in urea nitrogen and specific gravity of blood; blood chlorides are only slightly decreased. (Wohl, Burns, and Pfeiffer.[5])

increase of blood nonprotein nitrogen values.[11, 12] Likewise, shock due to wounds will show a similar picture.[13, 14] The following cases illustrate azotemia due to acute gastrointestinal hemorrhage.

CASE 2.—N. D., a white man, fifty years old, who for at least two years prior to admission to the Philadelphia General Hospital had a typical peptic ulcer history with pain relief from alkali powders. About a week prior to admission to the hospital, he vomited undigested blood (about one quart of material in all) and passed tarry stools. Examination showed an Italian man with a warm, dry, and faintly icteroid skin, who appeared acutely ill, but was not complaining of any pain. The blood pressure was 140 systolic, 80 diastolic. Physical examination was normal so far as revealing any pertinent signs of the underlying condition was concerned. The man was diagnosed as a bleeding peptic ulcer case. The blood count showed a rather marked secondary anemia, and the blood urea nitrogen was 40 mg. per cent on June 6, 1936. Another blood specimen taken on June 8, 1936, showed a urea nitrogen of 90 mg. per cent. Because of the rising blood urea nitrogen, secondary anemia and the presence of casts in the urine, a note by the interne suggested reconsidering the diagnosis, although he added that the clinical state did not fit in with the picture of chronic glomerulonephritis.

By June 9, 1936, the blood pressure was 116 systolic, 74 diastolic. The heart and lungs were negative. On a Sippy diet, the man continued to improve. By June 11, 1936, the blood pressure was 130 systolic, 74 diastolic, and he was considered fit under cyclopropane anesthesia for operation, which was performed June 11, 1936, and a duodenal ulcer was found.

By June 10, 1936, the patient's blood urea nitrogen was 15 mg. per cent, and on the day of the operation it was down to 12 mg. per cent. His convalescence was uneventful from every standpoint. The diagnosis of bleeding into the bowel remained the chief element associated with the cause for his admission and the immediate forerunner, if not the actual cause, of the azotemia.

CASE 3.—Mrs. D. B., a white woman, aged forty-nine years, admitted to Temple University Hospital with a chief complaint of fainting and weakness with vomiting of black material and passage of "pitch black stools" the day before admission Dec. 1, 1936. The vomiting was repeated once before admission.

The past medical history was irrelevant, including the gastrointestinal tract which was entirely negative up to the present illness. The patient was noted as one who was accustomed to endure hardships, to work hard and to bear many children. The physical examination being negative, a diagnosis of bleeding duodenal ulcer was made, based on the history. The blood pressure was 90 systolic, 65 diastolic. The blood count on admission was: erythrocytes 3,180,000; leucocytes 18,400; polymorphonuclears 86 per cent; lymphocytes 16 per cent. The urine showed a specific gravity 1,017, a trace of albumin with negative microscopic findings. The blood chemistry reported on the day after admission, Dec. 2, 1936, showed the urea nitrogen to be 88 mg. per cent and the blood chlorides to be 524 mg. per cent. The patient was given several blood transfusions. She improved markedly in general and so far as the blood count and the blood chemistry were concerned, the latter in three days (by Dec. 5, 1936) was decreased to 26 mg. per cent urea nitrogen. Her blood pressure on Dec. 7, 1936, was 190 systolic over 100 diastolic, and the eye-ground examination revealed a severe grade of arteriosclerosis. Occult blood was still present in the stool on Dec. 30, 1936, and continued to the time of discharge on Jan. 19, 1937, although the patient felt well, and the erythrocyte count was 4,450,000. She had been put on a Sippy diet. Her blood pressure on discharge Jan. 19, 1937, was 240 systolic over 136 diastolic, and she was put down definitely as a case of essential hypertension. Her stools remained positive for occult blood throughout her stay in the hospital, although proctoscopic examination revealed no signs of bleeding from hemorrhoids or other disease of the lower bowel. It should be added that an x-ray series on Dec. 15, 1936, was reported as definitely indicating a duodenal ulcer on the lesser curvature of the first portion of the duodenum.

Comments.—These two cases parallel closely those reported by Sucic[15] and Ingegno[10] in that high blood urea values are observed within a short time after obvious bleeding into the gastrointestinal tract. The hyperazotemia declines promptly on cessation of the bleeding. The persistence of a positive occult blood test in the stools in the last case is at variance with the impression of Ingegno, that continued bleeding results in persistence of elevated urea values and which, he, therefore, assumes to be of great prognostic value. The amount of actual bleeding in this case, it is true, may be questioned, as the blood count and other signs of hemorrhage did not speak for continued loss of appreciable quantities of blood into the bowel. It has been suggested that the elevation in blood urea may be due to increased liberation of the protein of the blood into the intestines and to resorption of blood proteins from the intestinal tract.

If this explanation approximates the truth, then one would expect at least some elevation of the blood urea in patients with ruptured ectopic pregnancy. With this point in view, we reviewed twenty case histories of patients who were operated upon at the Philadelphia General Hospital in the last two years (Table II). All the urea nitrogen values, it will be seen, were well within normal range.

It would seem, therefore, that the azotemia in hemorrhage is probably not conditioned by the resorption of blood. The loss of plasma and electrolytes through the intestinal tract would appear to be the more correct explanation. Continental writers refer to the condition of hyperazotemia of gastrointestinal hemorrhage as "exhaustio virium."[18] If it is unrecognized and not treated early by sufficient amounts of sodium chloride solution, the patient usually dies. Death in such instances, as generally held, is due to extrarenal uremia rather than to the severe anemia.

TABLE II

BLOOD FINDINGS IN CASES OF RUPTURED ECTOPIC PREGNANCY

CASE NO.	PROBABLE DATE OF RUPTURE	OPERATION DATE	UREA	BLOOD TAKEN DATE	ERYTHROCYTES	LEUCOCYTES	WASSERMANN
1	Old	2/ 2/36	13.0		4.3	9,600	++++
2	2/ 2/36	2/ 6/36	10.0	2/ 8/36			
3	11/12/36	11/23/36	10.0	11/19/36	4.1	10,600	Neg.
4	10/30/36	11/23/36	11.0	11/10/36	2.5	20,550	+
5	11/27/36	12/14/36	10.0	12/ 3/36	2.6	5,120	++++
6	?Bleeding	8/27/36	10.0	8/24/36	3.5	3,560	++++
7	6/16/36	6/17/36	14.0	6/18/36	2.4	10,300	
8	4/11/36	4/12/36	14.0	4/14/36	4.1	13,950	Neg.
9	7/17/36	8/27/36	10.0	8/24/36	3.5	12,000	+++
10	12/ 2/36	12/15/36	10.0	12/ 3/36	2.6	5,150	++++
11	11/ 9/36	11/23/36	11.0	11/10/36	2.4	17,900	++++
12	1/23/36	1/23/36	11.0	1/27/36	2.8	14,800	Neg.
13	3/11/36	3/12/36	15.0	3/16/36	3.6	12,550	Neg.
14	2/ 4/37	2/ 8/37	8.0	2/ 8/37	2.5	14,150	Neg.
15	2/ 6/37	2/ 6/37	10.0	2/ 6/37	4.1	14,500	Neg.
16	?	9/15/36	9.0	9/ 8/37	3.5	11,900	Neg.
17	10/30/36	11/ 7/36	8.0	11/ 9/36	2.5	20,500	+
18	6/ 3/36	6/ 3/36	13.0	6/ 3/36	3.6	7,200	Neg.
19	1/31/37	2/19/37	12.0	2/20/37	4.3	8,600	Neg.
20	1/27/37	1/27/37	15.0	1/23/37	2.9	17,900	Neg.

A very interesting case of a type not hitherto reported in the literature is that of unresolved bronchopneumonia treated by x-ray.

CASE 4.—A white man, E. W., aged 50 years, admitted to the Philadelphia General Hospital, Feb. 16, 1936, for cough and shortness of breath; the physical signs as well as the x-ray the following day indicated a confluent bronchopneumonia. The day after admission his blood urea nitrogen was 21 mg. per cent. On March 3, 1936, he began to have x-ray treatments of his lung on account of failure to resolve. By March 6, 1936, his blood urea nitrogen had risen to 105 mg. per cent. The urinalysis was acid; specific gravity 1.015; albumin 0; sugar 0. Microscopic examination negative. A Mosenthal test showed a specific gravity of 1.010 to 1.017. Blood count was 3,500,000; the blood Wassermann was negative. By March 16, 1936, his urea nitrogen was 36 mg. per cent and his blood chlorides were 605 mg. per cent. The patient developed erysipelas during convalescence, and on his return from the contagious disease hospital to which he had been transferred, he had a normal urinalysis and a blood count of 3,670,000 erythrocytes, 9,850 white blood cells, and a normal differential. The blood urea nitrogen was 20 mg. per cent.

Another similar case came under our observation at the Philadelphia General Hospital (service of Dr. William Egbert Robertson) of a young woman, thirty years old, who received x-ray treatments of her lung for an unresolved lobar pneumonia. Prior to the irradiation, the blood urea was 12 mg. per cent. Following the x-ray therapy, the blood urea had risen to 90 mg. per cent. The urinalysis, the Mosenthal test, and the eye-ground examination failed to implicate the kidneys as the cause of the azotemia.

A rise in blood urea nitrogen during the course of malarial therapy of dementia paralytica has been of frequent occurrence in patients at the Philadelphia General Hospital. In view of our interest in nonrenal azotemia, it appeared desirable to make a more critical study of these changes.

The records of two hundred and fifty cases of paresis admitted to the Philadelphia General Hospital between the years 1928 and 1937 were examined to determine the normal blood urea nitrogen for such a group, and also to note any changes in this finding that might occur following fever therapy, with particular reference to malaria. It should be noted here that many of these cases showed various other forms of syphilis or complicating systemic diseases.

All but sixteen of these 250 cases had a blood urea nitrogen of 20 mg. per cent or under, the average being 14 mg. per cent. Twenty milligrams per cent was considered as the top normal.

Ninety of the cases showing normal blood urea nitrogen figures were given a course of malarial therapy, averaging ten elevations of temperature. Of this group 35 cases (37.7 per cent) had urea nitrogen increases of varying degree, up to 110 mg. per cent, the average being 39 mg. per cent. Determinations of urea nitrogen were usually obtained biweekly in every case of paresis so treated. In analyzing these cases, it was observed that the urea rise might occur at the beginning, middle, or end of treatment, and, therefore, no prediction could be made of this possibility in any given case. It was concluded, however, that increases above 40 mg. per cent were of bad prognosis inasmuch as of seven of the patients who had such rise, three (43.0 per cent) died; while of the twenty-eight others whose rise in blood urea nitrogen was less than 40 mg. per cent, only five (17.5 per cent) died. No changes were observed in the plasma chlorides. In a few cases where plasma chlorides were determined at the time of a sudden urea rise, no abnormal depression of the former was noted. Other workers have reported some reductions in chlorides in patients undergoing malarial therapy.[17]

Comments.—Blood urea nitrogen frequently, though by no means always, is increased during the course of malarial therapy of paresis.

SUMMARY AND CONCLUSION

It is still not generally appreciated that high blood urea nitrogen values are found in many diverse clinical states other than chronic glomerular nephritis. In the majority of instances, the clinical and biochemical changes of the blood reflect loss of electrolytes and fluid through secretions from the gastrointestinal tract.

There are, however, other clinical states in which there is no such obvious loss of electrolytes and fluids. A series of such cases is described. It is to be stressed that the group of conditions associated with hyperazotemia and hypochloremia bear certain clinical and biochemical similarities. Likewise, they have many features in common with adrenal insufficiency, as was pointed out in our earlier publication. In the light of the recent work of Harrop on the salt-water hormone of the adrenal cortex, which controls sodium metabolism, and our findings of morphologic changes in the adrenals in hyperazotemia and

hypochloremia of high intestinal obstruction, it is strongly suggestive that the pathogenesis of the many similar cases of disturbed electrolyte and water metabolism, with associated hyperazotemia and hypochloremia, may find its proper explanation in a cortico adrenal insufficiency.

A special study was made of cases of dementia paralytica treated with malaria. In 37.7 per cent of such patients, there was demonstrable a nonrenal azotemia with an average of 39 and a maximum of 110 mg. per 100 c.c. blood.

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THE SOURCE OF URINARY INDOL*

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INDOL was recognized as early as 1875 as a product of bacterial decomposition of proteins. In 1901, Hopkins and Cole discovered tryptophan, and in 1903, these same authors demonstrated that tryptophan is the source of bacterially formed indol. It has been shown that tryptophan is the only constituent of the protein molecule which can be transformed into indol by bacteria.[1]

Since practically all proteins contain tryptophan and since the gastrointestinal tract is heavily ladened with the types of organisms which are most active in indol formation, it might be expected that indol would be formed in the intestine and pass into the blood stream. Under normal conditions this indol does not appear in the free state in the urine because of the efficient detoxifying mechanism which is available. Free indol, however, has been reported as present in the urine in various pathologic conditions.

The appearance of free indol in the urine might indicate a failure of the detoxifying mechanism which is presumably mainly localized in the liver. On that basis its determination in the urine could serve as a test for liver dysfunction. With this in mind Vaughan examined the urines from a series of patients suffering from various pathologic conditions of the liver and also from patients with severe putrefaction and intestinal stasis.[2] He was not able to find indol in any of the urines tested. The method employed was to precipitate bilirubin with barium chloride, extract the filtrate with petroleum ether, and test with Ehrlich's reagent.

Forbes and Neale,[3] using a different analytical method which involved two steam distillations and testing the final distillate with sodium b-naphthoquinone sulfonate, examined urines from patients presenting a variety of pathologic conditions. No indol was found in the urine from normal individuals, but it was found in significant amounts in many cases of chronic arthritis, pellagra, diabetes mellitus, tuberculosis, toxemia of pregnancy, congestive heart failure, and lobar pneumonia. Amounts of 0.05 mg. and more per liter were considered significant.

More recently Vaughan[4] has advanced the argument that the positive tests may in many cases be explained by bacterial contamination.

We have employed the method of Forbes and Neale and have confirmed the statement that the urine of certain individuals gives a positive test for indol. It is with a possible explanation of these findings and the action of pure cultures of bacteria on sterile urine filtrates that we shall deal in this communication. Our experiments were carried out in the summer of 1935, but publica-

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tion was delayed pending further work which is now in progress. Recent interest in the subject prompts the authors to report the results of the first part of the investigation at this time.

As indicated above we feel that indol in the body is always a product of bacterial action. For the ultimate source it only remains to determine the locus of indol-producing organisms whether they be in the intestinal tract, some focus of infection, or in the urinary tract. A knowledge of the type of organism is very important since the specificity for indol production varies greatly. It is also of paramount importance to know whether similar compounds other than tryptophan itself can serve as the mother substance.

Indol has been demonstrated by Ehrlich's method as a product of the action of *E. coli*, *Proteus vulgaris*, pseudodysentery, and true vibrios.[5] *E. coli* is most vigorous in its attack on tryptophan,[6] and the indol so formed is not decomposed by the bacteria—in fact, indol in a concentration of 1:1500 has bacteriostatic qualities against *E. coli*.[7] These organisms are abundant in the intestinal tract. It has been suggested that they may pass rather readily into the blood stream, and it is not inconceivable that the average urine specimen might contain them, particularly if the conditions in the urine were favorable for viability. *E. coli* has long been incriminated as an infecting organism of the urinary tract. Traut and Kuder reported this organism to be present in 90 per cent of cases of urinary infections during pregnancy.[8]

Woods[9] has investigated the action of *E.* coli on compounds other than tryptophan (a-amino b-indol propionic acid). No indol was formed, under aerobic conditions from b-indol aldehyde, b-indol carboxylic acid, b-indol acetic acid, b-indol propionic acid, or b-indol acrylic acid. There was 10 per cent production from b-indol pyruvic acid when ammonia was present. In our experience, when certain strains of these organisms are grown in sterile urine filtrates, the reaction becomes alkaline due, no doubt, to the formation of ammonia. Woods[6] has also shown that under anaerobic conditions *E. coli* converts tryptophan to indol propionic acid. In both instances the rate of indol formation was proportional to the amount of tryptophan present.

Saito[10] has reported the production of indol by the action of *E. coli* on d-indol lactic acid. The l-isomer under the same conditions did not form indol. Indol pyruvic acid also gave rise to indol but required fresh cultures of the organism. A medium containing asparagin and inorganic salts was employed in these studies.

It is evident, therefore, that if urine is contaminated with *E. coli* and contains tryptophan, b-indol pyruvic acid, or d-indol lactic acid, indol will be formed during incubation, provided conditions are optimum. With this in mind, we have attempted to determine whether this might be the explanation of the positive indol tests which we and others have obtained on specimens of urine.

It was first necessary to deal with sterile urine filtrates in order to be certain that no contaminants were present. This was particularly true since Forbes and Neale invariably used morning urine samples, which had been in the bladder for several hours, and apparently took no precaution against contamination. We felt that perhaps the patients' urinary tracts were infected with *E. coli* or that the bacteria gained entrance after the urine was passed. If the determinations

were not made promptly or if the bladder contained organisms, it might explain the positive tests of Forbes and Neale as will be shown presently.

In the experiments three pure cultures of *E. coli* were used, two of which were isolated from catheterized specimens from patients suffering with infections of the urinary tract. The third came from a sample of buttermilk obtained at a local dairy. All three were vigorous indol producers from sterile media containing tryptophan.

Normal urines were passed through sterile filtration systems, each specimen separately, the sterility of the filtrate determined, and the sterile filtrates inoculated from one of the pure cultures of *E. coli*. These inoculated filtrates were incubated for varying periods at 37° C. Control filtrates were retained in each experiment. Distillations were carried out on the filtrates according to the method outlined by Forbes and Neale. The results on the control filtrates were compared with the original distillation of the urine, performed at the time the specimen was received and in most cases within a few minutes after collection. Typical experiments taken from a rather large number are shown in Table I.

TABLE I

URINE FILTRATE STUDIES

SPECIMEN	CULTURE USED	ORIGINAL	INDOL IN SEEDED FILTRATE	INDOL IN CONTROL FILTRATE
HEC	B	trace	+ 96 hr.	trace 96 hr.
HEC	A	trace	+ 72 hr.	trace 72 hr.
10	7	trace	+ 6 hr. + 36 hr.	trace 36 hr.
9	7	-	+ 6 hr. + 24 hr.	- 24 hr.
13	A	-	+ 24 hr.	- 24 hr.
5	A	-	- 4 hr. + 8 hr. + 24 hr. + 75 hr.	- 75 hr.

trace = less than 0.5 mg. per liter. + = more than 0.5 mg. per liter.

The results of the entire series of experiments may be summarized as follows:

1. The organisms grew rapidly in the urine filtrates. The formation of a pellicle was noted in some of the cultures.

2. The pH increased in proportion to the time of incubation as is seen in contaminated urine which is allowed to stand.

3. None of the controls contained more indol than the original urine specimen.

4. All inoculated filtrates were strongly positive to the test for indol.

5. The indol test on inoculated specimens became positive within a short time (five to six hours), and further incubation did not increase the amount of indol formed.

We have noted, as did Vaughan, that specimens which were at first negative for indol became positive on standing. For example, a sample which did not give an indol test was allowed to stand for ninety-six hours, at which time it had become positive.

Cole[1] states that indol is not formed from tryptophan by *E. coli* in the presence of glucose. As an added check on our conditions, we have carried out a few experiments in which glucose was added to the urine before filtration. Typical results are given in Table II. It will be noted that indol was not formed in any of the filtrates which contained 0.2 per cent or more of glucose. One would not, therefore, expect to find indol in diabetic urines containing this much glucose, unless it is formed elsewhere in the body and is present in the urine merely because it has escaped conjugation. Forbes and Neale[2] report positive tests on diabetic urines, but they do not state how much glucose the urine contained, or whether that particular sample contained any at all. We have not as yet had an opportunity to examine urines from patients with diabetes.

TABLE II

URINE FILTRATE STUDIES

BOTTLE	CULTURE	TREATMENT	RESULT
1.	7	0.2% glucose	negative indol test
2.	7		positive indol test
3. (control)	uninoculated		negative indol test
All bottles incubated 20 hours at 25° C.			
BOTTLE	CULTURE	TREATMENT	RESULT
1.	7	0.3% glucose	negative indol test
2.	7		positive indol test
3. (control)	uninoculated		negative indol test
All bottles incubated 5 hours at 28° C.			

There is also the possibility that the indol obtained from the inoculated filtrates in which the organisms were growing rapidly was within the bacterial cells themselves, and not directly related to any substrate in the urine specimens. To eliminate this possible source of error, we have performed the following test. Culture A was grown on agar for forty-eight hours, washed off with isotonic salt solution, and diluted to 100 c.c. There were twenty thousand million organisms per cubic centimeter. This suspension, when used in the regular distillation, gave a negative indol test.

These experiments indicate, we believe, that normal urine contains a substrate from which *E. coli* can produce indol. The fact that we noted early indol production and that further incubation produced no increase indicates that the substance is present in small, but rather constant, amount. The short time interval before indol becomes detectable argues for the substrate being a simple compound, since apparently combined tryptophan must first be released from its combination. The formation of the necessary enzymes takes time, and indol does not appear for a considerable time; a much longer time than the five to six hours, which we have noted in our experiments.

This is a confirmation under somewhat different conditions, using a different analytical method, of the work of Vaughan.

According to the results of other investigators discussed above, this substrate may be either tryptophan, indol pyruvic acid, or d-indol lactic acid. Isolation in pure form and chemical identification will, of course, be the final

proof of whether the material is one of the compounds mentioned, or some indol derivative whose behavior with *E. coli* has not yet been studied. Further investigation along this line is now in progress.

SUMMARY

1. Free indol has been found in some urine samples and was invariably found in sterile urine filtrates which had been inoculated with pure strains of *E. coli*.

2. The addition of glucose inhibits this indol formation.

3. Studies of sterile filtrates point to the possibility of the presence of some indol precursor in normal urine.

4. It is suggested that this precursor is tryptophan or some closely allied indol derivative.

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STUDIES ON MENINGOCOCCUS BROTH FILTRATES*

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THE presence of a soluble exotoxin in broth filtrates of meningococci, and the use of this toxin in skin tests on human subjects was reported by Herrold and Traut[1] and by Ferry, Norton, and Steele.[2] Ferry[3] tested a series of children and attempted to immunize the positive reactors by graded injections of toxin. The same tendency to associate susceptibility to infection and a positive skin reaction to this toxin was shown by Kuhns,[4] who found that in patients recovered from the disease the skin test was negative. This relationship, however, is by no means proved, as was pointed out by Maxcy.[5] Most investigators, from Gordon[6] to the present time, seem to doubt the

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existence of such an exotoxin, and maintain, as do Maegraith,[7] and Malcolm and White,[8] that toxic factors present in such filtrates may be products of autolysis of the bacterial cell. Rake[9] also seems to favor this hypothesis.

The pathogenicity of endotoxins present in autolysates of meningococci for various laboratory animals, notably rabbits and mice, has often been demonstrated.[11] Malcolm and White[8] noted that rabbits develop a tolerance or immunity to these substances if gradually increasing doses are employed. Experiments with exotoxin have been less common. Ferry[10] reported that monkeys surviving the injection of this substance intraspinally were thereafter immune to infection with virulent meningococci. The greater part of his reported work with animals, however, deals with their passive protection by means of antitoxin, and not active immunization with the toxin. The present work was designed to investigate further the skin reactions of various groups of individuals to dilutions of meningococcus broth filtrates, and the immunizing action of these filtrates in mice.

The broth filtrate was prepared according to the directions in Ferry's original article.[2] Four strains of meningococci, belonging to Group I-III, which had been isolated from patients within the previous eight weeks, were used. As recommended by Kuhns[4] merthiolate 1:10,000 was added to the filtrate as a preservative. The skin testing material was made up in dilutions of 1:1,000 and 1:800 in saline, and a control provided consisting of Ferry's hormone broth in the same dilutions and containing the same proportion of merthiolate.

Two groups of normal controls were first tested by injecting 0.1 c.c. each of toxin and broth control intracutaneously in the skin of the forearm. The reactions were read at the end of twenty-four hours, and only those showing a zone of erythema greater than 1 cm. in diameter were considered positive. Twenty-two adults showed 86 per cent positive reactions, and 16 children gave 56 per cent positive reactions. Broth controls were uniformly negative, both in these and in subsequent experiments.

Twenty-four cases of meningococcus meningitis who had recovered within the past two years were tested. These individuals were clinically well at the time and varied in age from infancy to forty-five years. It was found that 76 per cent of these gave positive skin reactions, which is about what one would expect in a control group of comparable age distribution. Following this, 13 cases, ill or just recovering from meningococcus infection, were tested, and all but one were found to be skin test negative. However, when a series of cases sick with one of a number of other infections such as tuberculosis, rheumatic fever, scarlet fever, pneumonia, and undulant fever were tested, it was found that there was a great predominance of negative reactions. Of 34 such patients, only 9 or 26 per cent gave positive reactions, which is a marked reduction from the 74 per cent of the combined control groups. A comparison of the results is presented in Table I.

We attempted to correlate the presence of the organisms in an individual with a negative or a positive skin test. Throat cultures were obtained from

the 24 recovered cases mentioned above at the time the skin test was done, and smeared directly on warm North-gelatin agar plates. Two carriers were discovered, both of whom gave negative intradermal reactions. One carrier, however, not in this series of recovered cases, was later discovered who had a strongly positive skin test. It appears, therefore, that in these few instances there is no evidence pointing to an association of the carrier state with a negative skin test. The occurrence of a positive skin test in one patient with meningococcus meningitis also speaks against any such association.

As can be seen in Table I, the group showing the lowest per cent of positive skin reactions is the sick meningococcus cases. The somewhat similar findings in the control group sick with other diseases make it seem likely that

TABLE I

GROUP		NUMBER TESTED	TOTAL	PER CENT POSITIVE	
Control	adult	22	38	86	74
	child	16		56	
Recovered cases		24		76	
Sick meningococcus cases		13		8	
Control sick cases		34		26	

these results can be explained as a nonspecific phenomenon. It is possible, however, that in some cases patients sick or recovering from meningococcus meningitis might contain antibodies in their serum capable of neutralizing the toxin of the skin test dose and so avoid any reaction. Serum from two such patients, removed on four occasions, failed to have this effect, either when mixed with the toxin in vitro before injection in four individuals, or when injected the day before at the site of the skin test. No neutralization of the skin test dose could be demonstrated in animals. Thirty rabbits gave uniformly positive skin tests, and remained positive even after 15 of these animals had received 3 or more injections of antimeningococcus serum, or had been immunized with suspensions of live organisms or intravenous toxin in large and repeated doses. Moreover, in three of the meningitis patients followed for two months after discharge, the skin test became positive. Hence it would seem that it is not the presence of specific infection nor the production of antibodies, but rather a temporarily altered reactivity of the skin at the time of any acute infection that is responsible for the negative skin tests in these patients.

To determine whether the filtrate had any immunizing effect against doses of virulent meningococci in white mice, a series of ten experiments were carried out, the number of mice in each ranging from 6 to 10, with an equal number of controls. One hundred fifty mice were used in all. The filtrate was injected intraperitoneally in doses of 0.5 c.c. at four-day intervals, the mice receiving 2 to 2.5 c.c. in all, while the control animals received the same doses of hormone broth. The injections of filtrate had no toxic effect. From ten to twenty-two days after the last injection of filtrate or broth, the mice were given intraperitoneally 0.5 c.c. of a heavy saline suspension of meningococci washed from North-gelatin agar plates. The results in each of the

ten experiments are similar, the filtrate-treated mice were much less affected by the meningococcus suspensions. The combined results of eight experiments are summarized in Table II.

TABLE II

	NUMBER OF MICE	MORTALITY	CLINICALLY* SICK	MORBIDITY TOTAL PER CENT
Filtrate treated	46	5	3	17.3
Broth treated	42	25	13	90.4

*By "clinically sick" is meant marked ophthalmia, roughening of the fur, and prostration so that the animals do not move when prodded.

This protective effect of the filtrate was apparently temporary. As can be seen in Table III, two series of mice injected with organisms forty-two days after the last filtrate injection were not protected as completely.

TABLE III

	NUMBER OF MICE	MORTALITY	CLINICALLY SICK	MORBIDITY TOTAL PER CENT
Filtrate treated	16	11	6	68.9
Broth treated	13	12	1	100.0

Four mice that had recovered from an injection of live meningococci after treatment with filtrate were reinjected with meningococcus suspension sixty days later. Two animals died and two were sick, giving a total morbidity of 100 per cent.

These experiments suggest that broth filtrates of meningococci have some active immunizing action in white mice against suspensions of the live organisms. Since in skin tests on human beings, these filtrates seemed to be nonspecific, we believe that different substances are responsible for the skin reactions and the effects in mice. We realize that this series is by no means conclusive and requires further study.

Very little is known about the composition of filtrates of meningococcus broth cultures, and therefore the results obtained in skin tests or animal experiments may be due to any one of many hypothetical products. Rake[9] has made definite advances in separation and analysis of the antigenic complex of the meningococcus, but the substances that he studied were obtained from a much older culture than we used here. Ferry[3] and Kuhns[4] have shown that autolysates from meningococci failed to give skin reactions comparable to those obtained with broth filtrates, and have thus supported their belief that the filtrates contain a true toxin and not merely products of autolysis. We repeated these experiments as follows: Saline suspensions of meningococci grown on solid media for twenty-four hours were spun down, the supernatant fluid discarded, and the sediment placed in the ice box till cultures were sterile. The organisms were then resuspended in hormone broth, the suspension being made equal in density to a four-day growth of the same strain of meningococci in hormone broth. The living culture was then filtered, while the suspension of killed organisms was placed in the incubator for four days before being filtered. Merthiolate 1:10,000 was added to both products after filtering. Parallel skin tests with similar dilutions of the two products showed a good positive reaction with the broth culture filtrate, but a negative result with the autolysate. Such results,

obtained by various workers, though suggestive, are not final evidence in favor of Ferry's hypothesis of an exotoxin, since it is impossible to exactly reproduce the conditions, enzymatic and otherwise, present in a live broth culture. Although it seems unlikely that the substance responsible for the skin test is a product of autolysis, the possibility remains that it may result from the decomposition of the broth medium by the action of growing meningococci.

Through the kindness of Dr. Rake, we obtained some purified specific Type I polysaccharide, and were able to compare known dilutions of this substance with various dilutions of autolysates and filtrates by means of precipitin tests with specific Type I rabbit serum. This serum had previously been absorbed with Type II organisms. The precipitin tests were carried out in the manner described by Rake and Scherp.[9] All meningococcus broth filtrates used in our experiments gave positive precipitin tests, demonstrating the presence of the type specific polysaccharide. There were also detectable amounts of the polysaccharide in some, but not all, of the preparations of autolysate. It would seem, then, that Ferry's broth filtrate contains various, little understood, substances, among them at least one substance identified as the type specific polysaccharide of the meningococcus isolated by Rake and Scherp. The substance responsible for the skin reactions and its mode of production still eludes definite analysis.

CONCLUSIONS

(1) Skin reactions using dilutions of meningococcus broth filtrates, prepared according to the method of Ferry, seem to be nonspecific and of questionable diagnostic significance.

(2) A certain proportion of white mice can be immunized against doses of virulent meningococci by injections of a meningococcus broth filtrate.

(3) The nature of the substances present in meningococcus broth filtrates is probably varied and demands further investigation.

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CLINICAL INVESTIGATION OF INCREASED FRAGILITY OF THE ERYTHROCYTES*

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A DETERMINATION of the resistance of the erythrocytes to hypotonic saline solution has been done as a routine procedure on all blood dyscrasias admitted to the University Hospital. This fragility test has also been done in a large group of miscellaneous diseases as a possible diagnostic aid and in an attempt to compile more complete data concerning these various diseases. Since 1927, 1,750 fragility determinations have been made. This report is a brief analysis of those in which the fragility of the erythrocytes was found to be increased.

The technique which has been employed is that described by Todd and Sanford.[1] A stock solution of 0.5 per cent sodium chloride is carefully prepared and kept tightly stoppered. Uniform drops of this solution are added to a series of 12 tubes, so that the first contains 14 drops and the last one 25 drops. Using the same pipette, distilled water is added until each tube has a total of 25 drops. The dilutions thus obtained range from 0.28 per cent to 0.5 per cent sodium chloride, and by using a 1.0 per cent solution of saline, dilutions above 0.5 per cent can be prepared when desired. Venous blood is obtained in a clean, absolutely dry syringe, and a drop is placed in each tube. The tubes are inverted several times so that the blood and hypotonic saline are thoroughly mixed, and then are allowed to stand for two hours at room temperature. Beginning hemolysis is considered to occur in that tube in which the supernatant fluid is just tinged with hemoglobin, and complete hemolysis in the tube in which the "button" of unhemolyzed sedimented cells has completely disappeared. Other methods for determining the fragility of erythrocytes have been suggested.[2-4] Some of these may be more accurate, but are either too difficult or time consuming for use as a routine procedure.

Hemolysis when determined by this means normally begins at 0.44 per cent saline and is complete at 0.34 per cent. When it begins at 0.48 per cent, it is considered as definitely abnormal. In the 1,750 determinations, 126 patients showed an increased fragility. The clinical records of 111 of these patients were available and constitute the material for this analysis. Table I shows the diagnosis in each of these patients.

Twenty-five (22.5 per cent) of the 111 cases with increased fragility of the erythrocytes occurred in patients with myelogenous leucemia. Eleven (44 per cent) of these had hemolysis beginning at 0.5 per cent sodium chloride or higher, and hemolysis was complete at an average of 0.38 per cent saline, thus showing

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an increase in both beginning and complete hemolysis. During the period covered by the study, 129 cases of myelogenous leucemia were studied in this Hospital, so that 19.3 per cent had an increased fragility. That such a large percentage of the cases of myeloid leucemia would show an increased fragility was quite unexpected. None of these patients presented any clinical manifestations of hemolysis, so that excessive hemolysis apparently did not play a part in the production of the anemia in these patients. In a review of the recent literature, two references have been found dealing with changes in the fragility of the erythrocytes in myeloid leucemia. Daland and Worthley[4] found a decreased minimal resistance and an increased maximal resistance in seven cases of this disease, and Watkins,[5] in reporting some atypical blood dyscrasias, stated that he had found hemolytic features in the early stages of myelogenous leucemia.

TABLE I

DIAGNOSIS	NO. OF CASES
Myelogenous leukemia	25
Lymphoma	6
Pernicious anemia	7
Hemolytic icterus (congenital)	18
Hemolytic icterus (acquired)	11
Anemia of pregnancy	8
Hypochromic anemia	8
Myxedema	3
Polycythemia	2
Albers-Schönberg disease	1
Icterus neonatorum gravis	1
Infectious mononucleosis	1
Gaucher's disease	1
Monocytic leucemia	1
Acute leucemia	1
Lobar pneumonia	1
Carcinomatosis of bone marrow	3
Miscellaneous	13
	111

Although an increased fragility was common in leucemic myelosis, it was much less frequent in other types of leucemia. Only 4 cases of lymphatic leucemia were found with an increased fragility, all of which were in the chronic, slowly progressing form in adults. There was also one case of acute leucemia in childhood, and one case of monocytic leucemia showing these changes. Two other cases of lymphoma showed an increased fragility, one being a case of the lymphoblastic type, and one a lymphocytic type without leucemia. The increase in the fragility in this group was not as marked as was found in myelogenous leucemia (Table II). Gaisbrock,[6] in studying a wide variety of diseases, reported one case of lymphatic leucemia which showed no change in fragility, and Daland and Worthley[4] found no change in three cases.

Seven of the 111 cases had typical pernicious anemia. The hemolysis began on an average slightly higher than 0.48 per cent, and was complete at 0.34 per cent or above. During the period included in this analysis, 748 patients with pernicious anemia have been seen in this clinic, so there was increased fragility in only 0.093 per cent of the cases. Studies of the fragility changes in pernicious anemia by different investigators have resulted in many conflicting find-

ings. Hill[3] found an increased fragility in some instances, and a decrease in others. Daland and Worthley,[4] after studying 20 cases, found a normal maximal resistance and an increased minimal resistance of the erythrocytes. They also noted a marked decrease in minimal resistance during the period of reticulocyte crises. It has been shown by Daland and Zetzel,[7] and Mermod and Dock,[8] that the reticulocytes themselves are not consistently more easily hemolyzed by hypotonic saline than normal cells. According to these workers, changes in the resistance depend more on the size, shape, and hemoglobin content of the cells than on their age.

The fragility of the erythrocytes is characteristically increased in hemolytic icterus; this increase is usually quite marked, but varies from time to time dur-

TABLE II

DISEASE	VARIATIONS IN MIN. RESISTANCE	VARIATIONS IN MAX. RESISTANCE	AVERAGE MIN.	RESISTANCE MAX.	NO. CASES
Myelogenous leucemia	0.54 - 0.48	0.46 - 0.32	0.494	0.36	25
Lymphoma	0.50 - 0.48	0.40 - 0.32	0.483	0.375	6
Pernicious anemia	0.50 - 0.48	0.38 - 0.32	0.483	0.360	7
Anemia of pregnancy	0.52 - 0.48	0.40 - 0.32	0.492	0.360	8
Familial or congenital hemolytic icterus	0.54 - 0.48	0.44 - 0.32	0.509	0.398	18
Acquired hemolytic icterus	0.96? - 0.48	0.52 - 0.30	0.552	0.391	11
Hypochromic anemia	0.50 - 0.48	0.38 - 0.30	0.482	0.345	8
Carcinomatosis with bone metastasis	0.50 - 0.48	0.36 - 0.34	0.486	0.353	3
Polycythemia	0.54 - 0.48	0.30 - 0.30	0.510	0.300	2
Myxedema	0.48 - 0.48	0.38 - 0.30	0.480	0.366	3
Albers-Schönberg disease	- - -	- - -	0.52	0.36	1
Icterus neonatorum gravis	- - -	- - -	0.48	0.38	1
Infectious mononucleosis	- - -	- - -	0.50	0.38	1
Gaucher's disease	- - -	- - -	0.48	0.38	1
Acute leucemia	- - -	- - -	0.50	0.32	1
Monocytic leucemia	- - -	- - -	0.50	0.38	1
Lobar pneumonia	- - -	- - -	0.48	0.36	1
Miscellaneous	0.50 - 0.48	0.38 - 0.30	0.482	0.348	13
					111

ing the course of the disease, and occasionally is not present. In 18 of the 111 cases, the patients had typical manifestations of familial or congenital hemolytic jaundice. Here the variations from normal were quite marked, with both beginning and complete hemolysis occurring well above the normal values. The average figures given in Table II for beginning hemolysis are too low, since in some instances determinations were not carried above 0.5 per cent sodium chloride. Acquired hemolytic icterus, like the congenital or familial type, also shows a rather pronounced increase in the fragility of the erythrocytes, although this change is considered to be less marked and is more frequently absent than in the familial form. A diagnosis of acquired hemolytic icterus was made in 11 of our cases, and in this group the fragility changes were as marked as in the congenital or familial form.

The fragility of the red blood corpuscles is generally found to be either normal or decreased in hypochromic anemia. There were 8 typical cases of hypochromic anemia which had an increased fragility. These 8 cases were about equally divided into the idiopathic variety and that type brought on by chronic hemorrhage. The increase in the fragility was not marked, being above 0.48 per

cent saline in only one case. The diagnosis of anemia of pregnancy was made in 8 cases, and in all of these the anemia was of the hypochromic type. There was no significant reticulocyte increase, no increased van den Bergh reactions, nor any findings suggestive of either a hemolytic icterus or a primary anemia.

The final group is made up of 28 unrelated diseases. In the majority of these, hemolysis began at 0.48 per cent saline and was complete at an average slightly above normal. Three of these had carcinomatosis with bone marrow metastasis, and Waugh[10] has reported 2 similar patients with extensive carcinomatosis of the bone marrow who had a marked hemolytic anemia with an increased fragility. There were 3 patients with myxedema who had a fairly marked secondary anemia, and two with polycythemia vera. One case each of Albers-Schönberg disease, Gaucher's disease, infectious mononucleosis, and icterus neonatorum, presented a slight increase in the fragility of the erythrocytes. One patient with lobar pneumonia was found with a definite but slight increase in fragility, whereas Needles[11] has noted a definite decreased fragility in 30 cases of this disease occurring in the Amazon Valley.

CONCLUSIONS

It is well recognized that the erythrocytes of patients with hemolytic icterus are more easily hemolyzed by hypotonic saline than are those of a normal individual. That there may be similar hemolytic changes in other blood dyscrasias is not so well known. Nearly 20 per cent of the cases of myelogenous leucemia seen in this clinic since 1927 have shown an increased fragility. In addition to the fragility changes, several of them had a definite increase in the number of reticulocytes. The combination of increased fragility, reticulocytosis, and an enlarged spleen in an aleucemia myelosis, might certainly lead to a diagnostic error. One of the patients here reported had a leucocyte count of 6,800, an erythrocyte count of 2,300,000, hemoglobin of 39 per cent (Sahli), and a reticulocyte count of 10 per cent. Hemolysis in this case began above 0.5 per cent and was complete at 0.40 per cent sodium chloride. The diagnosis in this case, which was subsequently confirmed at necropsy, was possible only because the blood smear was definitely abnormal and revealed many myelocytes. Other blood dyscrasias may occasionally have an increased fragility at some time in their course, but occur less frequently than in myelogenous leucemia. In a small number of our cases of pernicious anemia, lymphoma, hypochromic anemia, and others, the fragility of the erythrocytes was found to be increased.

Fragility determinations on 1,750 cases were reviewed. Of these, 126 had an increased fragility with hemolysis beginning at 0.48 per cent sodium chloride solution or higher. The records of 111 of these were reported.

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THE EFFECT OF LARGE DOSES OF DIGITALIS ON STANDARD METABOLISM*

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THE determination of the minute volume of the heart in cases of cardiac decompensation has been associated with certain difficulties. As is well known the value of the minute volume (M.V.) is obtained by dividing the standand metabolism by the oxygen absorption per liter of blood, i.e., the arteriovenous oxygen difference. The determination of the latter by means of the method modified by Grollman[1] undoubtedly gives reliable values under normal physiologic conditions, but the results obtained in cases of cardiac decompensation must be regarded with great skepticism. For many years we have often obtained in such cases inconstant results which have neither been due to defective gas analytical technique nor to the patient's not having been able to make the prescribed deep respirations. As an example, a case is here presented where almost daily determinations of the utilization were made for a long period, and where a fully satisfactory technique was employed; but in spite of everything, there was considerable variation in the results obtained (Table I).

It is difficult to say upon what these great variations depend. One might consider an inconstant mixing of alveolar air or possibly variation in blood flow in different parts of the lungs in cardiac decompensation, and especially with pulmonary congestion, something which probably is not met with to any considerable extent under normal physiologic conditions. Sonne and Nielsen,[2] however, consider that they have observed the first-mentioned even under certain normal physiologic conditions. The great variations in determinations of the minute volume, particularly in cardiac decompensation which are found in the literature, may be explained by the fact that it is difficult to exactly determine the arteriovenous oxygen difference. It is obvious that it would be difficult to arrive at reliable results when it is a question of following the influence of digitalis on the minute volume and stroke volume, respectively, in cases of cardiac decompensation. And, as has been said before, the results given in the

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literature must, therefore, in many cases be looked upon as uncertain. Nevertheless, both Grassman and Herzog[3] and Stewart and Cohn[4] consider that they have found an increase in the minute volume in these cases. Grassman and Herzog obtained, however, the same results with both the Grollman method and the Broemser method.

For several years we[5] have, like others,[6, 7] made the observation that as a rule the standard metabolism is increased in cases of cardiac insufficiency; but as the insufficiency improves a successive decline in the standard metabolism takes place. There is, in certain cases of cardiac insufficiency, a very considerable increase in oxygen consumption during rest and under fasting conditions with relative values of plus 50 per cent and over, values comparable with those met with in hyperthyroidism.

TABLE I

DETERMINATIONS MADE OVER A CONSIDERABLE PERIOD OF THE UTILIZATION OF OXYGEN IN A CASE OF CARDIAC DECOMPENSATION

DATE	ARTERIOVENOUS OXYGEN DIFFERENCE. C.C. O_2 PER LITER OF BLOOD	MEAN VALUES	GREATEST VARIATIONS
5/26/32	99, 87, 86	91	13 c.c.
5/27/32	116, 103, 105, 108, 85	103	31 c.c.
5/28/32	86, 70, 86, 94	84	24 c.c.
5/30/32	67, 114, 114	98	47 c.c.
5/31/32	116, 96	106	20 c.c.
6/ 2/32	94, 82, 60	79	34 c.c.
6/ 3/32	73, 88, 76	79	13 c.c.
6/ 4/32	94, 107	101	13 c.c.
6/ 7/32	81, 72	76	11 c.c.
6/ 9/32	73, 93, 89	85	20 c.c.
6/10/32	96, 74, 87	86	22 c.c.
6/13/32	90, 125, 72, 91, 90, 115	94	53 c.c.

For the more intensive study of the therapeutic effect of digitalis in the treatment of cardiac decompensation, a series of determinations of the standard metabolism in a number of cases of typical cardiac insufficiency was made, therefore, very soon after the admission of these patients to the clinic, both before and at a definite time after the intravenous injection of large doses of a new preparation of digitalis, "Digiton Leo," which contains both digitalis lanata and purpura glucosides. The investigations were made under strictly standard conditions, with the patients in a semirecumbent position. Krogh's spirometer was used for determining the standard metabolism, and Recklinghausen's grypotonograph for determining the blood pressure. The heart rate was determined by means of electrocardiography.

The material comprises 12 cases. The doses of "Digiton" varied from 500 to 1000 frog doses, which would correspond to 0.25 to 0.50 grams of digitalis leaves. In 9 cases there were grave signs of heart insufficiency with dyspnea, anasarca, and considerable enlargement of the liver (Cases 1, 2, 3, 4, 5, 6, 9, 11, and 12). In all these cases extreme enlargement of the heart could be established at the same time. After treatment with "Digiton" and with "Hydrargan Leo" (salyrgan), the edema disappeared in all the cases, with a larger or smaller

decrease in weight. Consequently, the liver was not palpable when the patients were discharged, except in Cases 6, 9, and 11, in whom there was cirrhosis of the liver. Cases 7 and 8 should probably be included in this group representing grave cardiac insufficiency, in spite of the absence of edema and acute swelling of the liver. In the last-mentioned cases, the liver proved to be considerably enlarged but showed no tendency to decrease in size with treatment. Case 10 lacked all signs of cardiac insufficiency during rest and was the only one out of all the cases with a normal-sized heart. In spite of this, he had dyspnea on exertion and showed an obvious insufficiency, with a considerably increased relative oxygen debt in the so-called "stair test." In all the cases with the exception of Cases 9 and 10, the standard metabolism was increased before the injections. As has been mentioned, Case 10 lacked signs of insufficiency during rest. The low standard metabolism of +7 per cent in Case 9 is noteworthy.

In six of the cases (1, 2, 3, 4, 5, and 12) an obvious effect from strong digitalization of the patients was observed only momentarily. Before the injection, the standard metabolism is, as stated before, already increased; but almost immediately after injection it rises to a higher degree. This further increase in the standard metabolism was gradually followed by decreased oxygen consumption, so that in Cases 3, 4, 5, and possibly 12, within an hour after the injections, the standard metabolism lay considerably below the initial value before injection. In five cases, where examinations were made several days in succession, the initial values the day following injection were lower than on the previous day and were followed by a renewed increase within a certain time after the injections. This appears particularly clear in Cases 1, 2, and 12. The mean value of the standard metabolism in these six cases (1, 2, 3, 4, 5, and 12), at the beginning of the investigations exceeded the normal value by 42 per cent. and after the injection of "Digiton" was followed by a mean maximum value of +65 per cent, subsequently with a mean minimum value of +28 per cent. In these six cases of severe cardiac decompensation without cirrhosis of the liver, the maximal momentary increase in oxygen consumption after injection of "Digiton," on the average, amounted to 47 c.c., with a mean error of ±7 c.c. In these cases it is not only the standard metabolism which is momentarily increased after the injection of "Digiton" but also the systolic blood pressure. (In Case 4 the blood pressure was not determined.) It is striking that at the beginning of the investigation all the cases showed auricular fibrillation with a heart rate of 100 or over. After several daily administrations of "Digiton," there was in all cases a considerable fall in the ventricular rate to 40 to 50 per minute. Also in all the cases already mentioned (1, 2, 3, 4, 5, and 12), the daily treatment with "Digiton" resulted not only in decreasing standard metabolism but also in simultaneous improvements in the patients with considerable diuresis, loss of weight, and disappearance of the swelling of the liver.

In the remaining six cases (6, 7, 8, 9, 10, and 11), the effect of daily administrations of digitalis intravenously on the standard metabolism is more doubtful or entirely absent; but it should be mentioned that these cases were not followed as closely as the former group. Attention should, however, be

called to the remarkable fact that in all of them there was cirrhosis of the liver, except in Case 10 in which insufficiency during rest was not present. In these cases the liver showed no tendency to decrease in size in spite of intensive digitalis therapy combined with mercury diuretics. Signs of a decrease in heart rate were also absent except in Case 9 where there was some decrease. It is also striking that only this latter case exhibited auricular fibrillation.

DISCUSSION

As in a number of both older and more recent investigations, results are here presented which clearly prove that there is a raised standard metabolism in typical cases of cardiac insufficiency. The mean values for the 11 cases at the first examination amount to a mean of +40 per cent. The explanation of this increased oxygen consumption is disputable. Eppinger[8] has found an increased lactic acid content in the blood in cardiac insufficiency even during rest; but above all, after work. This latter condition has been verified among others by Jerwell,[9] Meakins and Long.[10] A deficient resynthesis takes place with an increased oxygen consumption (Hill's "oxygen debt"). It would, therefore, be of the greatest interest to investigate whether or not there is any parallelism between the increased standard metabolism and the lactic acid concentration in the blood, but such an investigation would probably call for the puncture of the right auricle, for it is not certain that the venous blood from the extremities and from the portal system have the same lactic acid concentration. On the contrary, it is possible that it is the amount of congested blood in the liver, caused by cardiac insufficiency in certain cases, which is responsible for the increased oxygen consumption.* The momentary further increase in the standard metabolism, called forth by means of "Digiton," might perhaps be explained in this way. As far as can be judged from this investigation (the material being still small in amount), this momentary increase in the standard metabolism due to treatment with "Digiton" takes place, on the whole, only in the cases where there is acute congestion of the liver; but, on the other hand, not in those cases of insufficiency which are associated with cirrhosis of the liver. (It is possible that some of the cases labeled cirrhosis may have been due in some degree to congestion.)

In such recent works as that by Grollman, Möller[11] and Weese,[12] it is fairly definitely assumed that the minute volume of the heart is increased by digitalis, which is extremely probable since the standard metabolism and the blood pressure increase. The result is that there is a considerable increase in the stroke volume because the cardiac frequency decreases so appreciably. This latter circumstance should lead to a momentary increase in the volume of the heart also, a matter about which, however, we have no knowledge. As has been mentioned we have observed after injections of "Digiton," at first a momentary increase followed later by a considerable decline in the standard metabolism, a falling blood pressure, probably followed by a decrease in the minute volume, stroke volume, and heart volume. For example, in Case 12 a decrease in the size of the heart even up to 400 c.c. appeared.

*It would have been of interest to determine the standard metabolism under the influence of morphine while the oxygen-increasing tendencies of the respiratory muscles were diminished.

CONCLUSIONS

1. In cases of considerable cardiac insufficiency with congestion, swelling of the liver, and anasarca, the standard metabolism has been found to be appreciably increased.

2. In cases without cirrhosis of the liver, "Digiton" in large doses gives rise to a momentary further increase in the standard metabolism and blood pressure, and a reduction in the heart rate, following later by a considerable reduction in the standard metabolism and blood pressure. Associated with these changes there is an improvement in the patient, with reduction in the size of the swollen liver and considerable relief from edema.

3. In cases of cardiac insufficiency with cirrhosis of the liver, digitalization does not seem to cause a momentary increase in the standard metabolism.

CASE REPORTS

CASE 1.—Thirty-nine-year-old male coach painter: Cardiac decompensation with syphilis (mitral insufficiency and myocardial degeneration). Electrocardiogram indicates the possibility of coronary disease.

History.—Patient was a rather keen sportsman and football player. He was perfectly healthy until the middle of April, 1935. Then he began to feel shortness of breath and had palpitation when he walked upstairs. He was unaware of any venereal infection. Previously he had been treated at the clinic for cardiac decompensation in 1935. He again received treatment at the clinic from Dec. 27, 1935, to Feb. 25, 1936.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
12/27/35						71	
12/28/35	Before injection	303	+28	125/98	138		400
	23 min. after injection of 500 F.D.* "Digiton Leo"	328	+39	140/82	108		
	50 min. after injection	311	+32	—	108		
12/29/35							2300
12/30/35	Before injection of 500 F.D. "Digiton Leo"	255	+ 8	125/78	108		
	15 min. after	276	+17	135/80	78		3300
	30 min. after	276	+17	135/85	74		
12/31/35	Before injection of 500 F.D. "Digiton Leo"	227	± 0	110/75	72	66	
	15 min. after	253	+12	140/82	72		
	30 min. after	253	+12	140/80	66		
1/ 1/36							2000
1/ 2/36	Before injection of 500 F.D. "Digiton Leo"	253	+12	125/80	84		
	15 min. after	261	+15	125/80	72		1300
	30 min. after	269	+19	120/80	68		
1/ 3/36	Before injection of 500 F.D. "Digiton Leo"	252	+15	115/70	72	63	
	15 min. after	251	+15	118/72	68		
	30 min. after	242	+10	120/72	54		

*Frog doses.

Examination on Admission.—General condition fairly good. Height 171 cm.; body weight 71 kg. Temperature afebrile. Sedimentation rate (Westegren), 5 mm. per hour. Urine 0. Wassermann positive. Thyroid not palpable.

Examination of the Circulation.—Physical Examination: Extensive edema of the legs, involving also the undersides of the thighs, and pronounced sacral edema. Slight general cyanosis, and slight yellow coloring of the sclerae. Meulengracht index of the blood serum 1:15. Liver palpable one hand's-breadth below the costal arch. Veins of the neck were greatly distended when the upper part of the body was at an angle of 30°. Circulation time measured by the decholin method, 48 seconds. Blood pressure 110/70. Circumference of the abdomen 92 cm. *Heart:* Apex impulse palpable at the fifth interspace just outside the midclavicular line, diffuse and somewhat heaving. Prolonged apical systolic murmur, sounds distant.

X-ray of the Heart.—Heart measured 18.5 cm. in length, 14 cm. in width, and 12.5 cm. in sagittal direction. Volume about 1,360 c.c., corresponding to 780 c.c. per square meter body surface. Heart showed enlargement mainly to the left and posteriorly. Pronounced pulmonary congestion. On the right side there was a slight effusion in the pleura.

Electrocardiogram.—Rhythm irregular. Rate 140 per min. QRS interval 0.07. T-wave slightly upright in all leads. Deflections fairly small and ventricular complexes slightly notched in all leads.

Diagnosis.—Auricular flutter 2 to 1, 3 to 1.

Course.—Patient was sent to bed and put on 1,000 c.c. fluid daily. The day after admission, Dec. 27, the first determination of the standard metabolism was made. Liver was not palpable when the patient was discharged.

CASE 2.—Fifty-seven-year-old male coal shoveler: Severe cardiac insufficiency (mitral insufficiency and myocardial degeneration).

Physical Examination on Admission Feb. 11, 1936.—Marked edema with liver enlargement. Pulse deficit 28 per min.

X-ray of the Heart.—Pulmonary congestion. Heart volume 800 c.c. per square meter of body surface.

Electrocardiogram.—Auricular fibrillation.

Course.—Lost 7.4 kg. weight during treatment. Liver slightly palpable when discharged.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
2/12/36	Before injection of 1000 F.D. "Digiton Leo"	281	+49		120	57.3	1000
	15 min. after	272	+43		70		
	30 min. after	339	+79		60		
2/13/36	Before injection of 1000 F.D. "Digiton Leo"	196	+ 4	125/95	80		
	15 min. after	213	+13	140/90	50		1100
	30 min. after	230	+22	130/85	40		
	35 min. after	256	+35				
2/14/36		214	+ 8			54.6	

CASE 3.—Sixty-six-year-old male brewer: Severe cardiac insufficiency (mitral insufficiency and myocardial degeneration).

Physical Examination on Admission Feb. 24, 1936.—Severe edema. Liver enlargement.

X-ray of the Heart.—Marked enlargement, 2,000 c.c.

Electrocardiogram.—Auricular fibrillation and partial bundle branch block.

Course.—Loss of weight up to March 6, 3.5 kg. Liver not palpable at time of discharge.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
2/25/36	Before injection of 1000 F.D. "Digiton Leo"	312	+37	160/128	110	82.5	800
	Immediately after	407	+79				
	14 min. after	416	+82		100		
	28 min. after	404	+77	180/ 90	90		
	48 min. after	321	+41		80		
	60 min. after	377	+65	175/105	80		
	75 min. after	328	+44	180/120			
	100 min. after	268	+18	180/120			
2/26/36	Before injection of 1000 F.D. "Digiton Leo"	303	+33	160/115	90	82.4	2300
	7 min. after	258	+13		60		
	15 min. after	314	+38		58		
	30 min. after	266	+17	180/105			
	40 min. after	258	+13	170/105			
2/27/36	Before injection of 1000 F.D. "Digiton Leo"	244	+ 9			80.4	2150
	Immediately after	240	+ 7				
	40 min. after	209	- 7				

CASE 4.—Sixty-seven-year-old male farmer: Severe cardiac insufficiency (hypertension and myocardial degeneration).

Physical Examination on Admission.—Slight edema, marked enlargement of the liver.

X-ray of the Heart.—Generalized cardiac enlargement; total volume, 1,640 c.c.

Electrocardiogram.—Auricular fibrillation and left axis deviation.

Course.—Lost 5 kg. weight during first two weeks. Liver not palpable at time of discharge.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
3/1/36	Before injection	355	+76	190/115	110	70	
	Injection of 1000 F.D. "Digiton Leo"						
	3 min. after	384	+91	205/110			
	13 min. after	393	+95				
	30 min. after	360	+78	210/105	90		
	42 min. after	317	+57		100		
3/2/36	Before injection of 750 F.D. "Digiton Leo"	317	+57		80	70	500
	Immediately after	301	+49				
	15 min. after	256	+27		60		

CASE 5.—Sixty-six-year-old male watchman: Cardiac insufficiency (hypertension, myocardial degeneration, and coronary disease).

Physical Examination on Admission.—Slight edema, marked enlargement of the liver.

X-ray of the Heart.—Marked enlargement of the heart, 2,100 c.c., or 1,100 c.c. per square meter of body surface.

Electrocardiogram.—Auricular fibrillation, bundle-branch block, and ventricular extrasystoles.

Course.—Loss of 1.2 kg. in weight. Liver slightly palpable at time of discharge.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
3/4/36	Resting before injection	328	45		70	78.5	1400
	Immediately after injection of 1000 F.D. "Digiton Leo"	320	44				
	20 min. after	345	55		50		
	45 min. after	268	21		50		

CASE 6.—Forty-five-year-old male barber: Cardiac insufficiency with cirrhosis of the liver.

Physical Examination on Admission, March 10, 1936.—Severe anasarca, ascites, and liver enlargement.

X-ray of the Heart.—Pulmonary congestion, generalized cardiac enlargement, 1,640 c.c., or 1,300 c.c. per square meter of body surface.

Electrocardiogram.—Normal.

Course.—Weight loss 25 kg. No change in size of the liver.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
3/11/36	Before injection of 1000 F.D. "Digiton Leo"	381	+24	128/60	100	107.8	400
	10 min. after	356	+16	133/60	90		
	30 min. after	309	± 0		90		
	45 min. after	326	+ 6	125/65	90		
	90 min. after	360	+17				
3/12/36	Resting before injection of 1000 F.D. "Digiton Leo"	467	+52		110	108.0	600
	3 min. after	492	+60		100		
	10 min. after	488	+58		100		
	30 min. after	463	+50		100		

CASE 7.—Forty-four-year-old male engineer: Cardiac insufficiency with cirrhosis of the liver (mitral and aortic insufficiency, myocardial degeneration, and coronary disease).

Physical Examination on Admission.—No edema, enlargement of liver, pulmonary congestion.

X-ray of the Heart.—Pulmonary congestion, generalized cardiac enlargement, 1,500 c.c. (850 c.c. per square meter of body surface).

Electrocardiogram.—Partial auriculoventricular block, bundle-branch block, ventricular extrasystole.

Course.—No change in size of the liver. Loss of weight 2 kg. at time of discharge.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.
4/9/36	Before injection of 750 F.D. "Digiton Leo"	312	+48	135/70	84	60.5
	8 min. after	341	+62	160/85	84	
	20 min. after	328	+55	170/90	84	
	40 min. after	320	+52	155/85	84	

CASE 8.—Seventy-two-year-old male electrician: Cardiac insufficiency with cirrhosis of the liver (mitral and aortic insufficiency, myocardial degeneration, and coronary disease).

Physical Examination on Admission.—No edema, moderate enlargement of the liver.

X-ray of the Heart.—Severe pulmonary congestion, generalized cardiac enlargement, 2,100 c.c. (1,250 c.c. per square meter of body surface).

Electrocardiogram.—Partial auriculoventricular block and bundle-branch block.

Course.—Weight loss 9 kg. No change in size of liver at the time of discharge.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
4/14/36	Resting before injection of 875 F.D. "Digiton Leo"	324	+73	148/53			63
	10 min. after	315	+69	158/53			
	21 min. after	306	+64				
	44 min. after	352	+88	145/48			

CASE 9.—Seventy-year-old male cobbler: Cardiac insufficiency with cirrhosis of the liver.

Physical Examination on Admission.—Slight edema, ascites, marked liver enlargement.

X-ray of the Heart.—Generalized cardiac enlargement, 1,800 c.c. (1,050 c.c. per square meter of body surface).

Electrocardiogram.—Auricular fibrillation, partial bundle-branch block.

Course.—No diminution in size of the liver. Loss of 7.4 kg. weight.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
3/16/36	Before injection of 750 F.D. "Digiton Leo"	211	+ 7	145/ 95	60	68.4	600
	10 min. after	207	+ 5	150/102	40		
	30 min. after	164	-17	145/ 95	50		

CASE 10.—Forty-nine-year-old male carpenter: Dyspnea on exertion.

Physical Examination on Admission.—No edema, no liver enlargement.

X-ray of the Heart.—Normal heart volume, 450 c.c. per square meter of body surface.

Electrocardiogram.—Left axis deviation.

Heart Function Tests.—Nylin's "Stair Test" showed marked insufficiency with slight test.

Course.—Loss of body weight, 3.05 kg.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.
4/21/36	Before injection of 750 F.D. "Digiton Leo"	286	+14	120/75	72	85
	10 min. after	257	+ 2	118/78	66	
	22 min. after	252	± 0	110/72	72	
	40 min. after	278	+11	120/82	72	

CASE 11.—Fifty-seven-year-old male carpenter: Marked cardiac insufficiency with cirrhosis of liver (mitral insufficiency, and coronary disease).

Physical Examination on Admission.—Marked edema of the legs, marked enlargement of the liver.

X-ray of the Heart.—Generalized cardiac enlargement, heart volume 1,480 c.c. (900 c.c. per square meter of body surface).

Electrocardiogram.—Bundle-branch block.

Course.—Loss of weight 7 kg. No change in size of liver at time of discharge.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.
3/21/36	Before injection of 1000 F.D. "Digiton Leo"	248	+34	120/90		
	10 min. after	242	+31			
	20 min. after	250	+35	130/95		
	40 min. after	267	+44	140/95		
3/23/36	Before injection of 1000 F.D. "Digiton Leo"	264	+43	120/80	84	
	10 min. after	267	+44	128/90	78	
	20 min. after	276	+49	120/80	72	
	40 min. after	267	+44	125/85	72	

CASE 12.—Forty-one-year-old trolley car operator: Severe cardiac insufficiency (mitral insufficiency and myocardial degeneration).

History.—History of acute polyarthritis in 1911 but following this he was healthy and worked hard. Dyspnea since Feb., 1936. Edema of the legs since three weeks before admission.

DATE		STANDARD METABOLISM IN C.C. O_2 PER MIN.	B.M.R. %	BLOOD PRESSURE	HEART RATE	WT. KG.	DIURESIS PER 24 HOURS
5/26/36	Before injection of 1000 F.D. "Digiton Leo"	282	+18	120/100	80	75.5	
	25 min. after	321	+37	145/100	90		
	40 min. after	273	+14	140/ 98	90		500
5/27/36	Before injection of 750 F.D. "Digiton Leo"	230	- 4	135/ 80	70		
	25 min. after	277	+15	140/ 85	90		
	40 min. after	267	+11	145/ 80	100		1100
5/27/36					36		
5/28/36	Salyrgan (2 c.c. intravenous)						5600
5/29/36	Before injection of 500 F.D. "Digiton Leo"	212	- 5	148/ 80	60		
	20 min. after	234	+ 5	150/ 88	60		
	40 min. after	243	+ 9.5	148/ 80	70		
	60 min. after	243	+ 9.5	165/110	60	69.4	1700

Physical Examination on Admission May 25, 1936.—General condition bad. No temperature. Sedimentation rate (Westegren) normal. Weight 75.5 kg. Thyroid not enlarged. Urine: albumin ++. Marked edema over nearly the entire body. Ascites. Liver markedly enlarged. Blood pressure 140/75.

Heart: Apex impulse felt in fifth interspace outside the midclavicular line. Rough apical systolic murmur. Apical first sound accentuated, also second pulmonic sound. No pulse deficit.

X-ray of the Heart.—Pulsations of the heart were small. Length of heart, 23 cm.; breadth, 19 cm.; saggital diameter, 16 cm.; generalized enlargement of the heart with bulging especially over region of left auricle. Pulmonary congestion.

Electrocardiogram.—Auricular fibrillation, partial bundle-branch block.

Course.—Weight loss, 20 kg. No liver enlargement at time of discharge.

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NONHEMOLYTIC STREPTOCOCCIC MENINGITIS: REPORT OF A CASE SUCCESSFULLY TREATED WITH SULFANILAMIDE AND PRONTOSIL

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SINCE the initial work by Domagk[1] on the chemotherapy of streptococcic infections with sulfanilamide, numerous other workers in this field have reported cases successfully treated with this drug, although no case of nonhemolytic streptococcic meningitis has heretofore been recorded. Among the significant papers are those of H. J. Gray,[2] who reviewed the literature on streptococcic meningitis up to July, 1935; Caussé, Loiseau, and Gisselbrecht,[3] who reported a case of hemolytic streptococcic meningitis with recovery after the use of prontosil; Buttle, Gray, and Stephenson;[4] Long and Bliss,[5] whose paper contains a notable bibliography on para-amino-benzene-sulfonamide and its chemical derivatives; Proom,[6] and Anderson,[7] who likewise reported a case of hemolytic streptococcic meningitis which recovered rapidly when prontosil was given by mouth and intramuscularly; Mellon, Gross, and Cooper,[8] who reported on their experimental studies with sulfanilamide in mice; and Weinberg, Mellon, and Shinn,[9] who reported two cases of meningitis, both of hemolytic streptococci, and both recovered on sulfanilamide and prontosil.

Of particular interest is the statement by Anderson[7]: "The concensus of all the authors seems to be that prontosil is of value in the treatment of hemolytic streptococcus infection, but it apparently has little or no effect on other strains of streptococci or other organisms."

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Reference should also be made to the Report of the Council on Pharmacy and Chemistry on Sulfanilamide and Related Compounds,[10] May 29, 1937, and particularly to the statement, "No success has been reported for the treatment of infections due to alpha and non-hemolytic streptococci . . ." with sulfanilamide.

The purpose of this paper is to report a case of nonhemolytic streptococcic meningitis of marked severity which has been successfully treated by the use of sulfanilamide and prontosil. The organism was gamma nonhemolytic streptococcus.* It produced no hemolysis in aerobic or anaerobic culture. It was gram-negative. Successive cultures of the organism grew well on blood agar, but no hemolysis occurred. Culture plates of *Streptococcus viridans* and hemolytic streptococci, for comparison, showed typical growths.

This case is of further interest because of the large amount of sulfanilamide which was given. Toxic symptoms resulted from this large dosage, but recovery took place promptly and without ill effects. Effort has been made to

TABLE I

DATE	SMEAR	CULTURE	CELL COUNT	SP. FL. SUGAR	SULFANILAMIDE IN SP. FL. (PER C.C.)
5/25/37	Positive	Positive	1,100		
5/26	Positive	Positive	2,660		
5/27	Positive	Positive	3,360		
5/28	Positive	Positive	3,680	5.4 mg.	
5/29	Positive	Positive	3,995	3.0 mg.	
5/30	Positive	Positive	2,220	6.1 mg.	
5/31	Positive	Positive	2,905	15.6 mg.	
6/ 1	Positive	Positive	3,550	10.9 mg.	Positive
6/ 2	Positive	Positive	3,120	7.8 mg.	Positive
6/ 3	Negative	Negative	1,472	12.8 mg.	Positive
6/ 4	Positive one chain	Positive	908	14.7 mg.	0.35 mg.
6/ 5	Negative	Negative	881	18.6 mg.	0.3 mg.
6/ 6	Negative	Negative	292	23.2 mg.	0.125 mg.
6/ 8	Negative	Negative	775	18.3 mg.	
6/10	Negative	Negative	502	21.7 mg.	
6/14	Negative	Negative	93	18.4 mg.	

In this table there is no correlation between the spinal fluid sugars and the blood sugar inasmuch as it was not feasible to take blood sugars at this time.

correlate the clinical and chemotherapeutic findings by qualitative and quantitative estimations of the amount of the drug in the spinal fluid, together with estimations of the spinal fluid sugar, cell counts, etc. (Table I).

The total quantity of sulfanilamide given by all routes was 1,164 grains. In this case the most effective method of introducing it was by rectum, because much of that given by mouth was promptly vomited (not included in total dosage), and there seems to be no good reason for introducing it intravenously. Intramuscular injections of 2.5 per cent prontosil were started when the diagnosis was made, and this was followed by oral and rectal administration. None was administered directly into the cistern or the spinal canal.

In addition to the drug therapy, cisternal and lumbar punctures were done as seemed advisable, the drainage each time being sufficient to lower the excessive pressure. Blood transfusions were given on four occasions. Glucose in

*I am indebted to Dr. R. F. Sledge, Lieut. Com. M. C., U. S. Navy for the bacteriologic work in this case.

saline was given as necessary to combat carbohydrate deficiency, acidosis, and water loss. It is, however, the distinct clinical impression that these were but secondary considerations in the recovery and that most credit for the successful outcome was due to the para-amino-benzene-sulfonamide. When the drug was given by rectum, 30 gr. of the powder was dissolved in 2 oz. of warm saline solution, thereby increasing the concentration the patient received from 2.5 per cent to approximately 4 per cent.

CASE REPORT

History.—J. C., a white female, aged six and one-half years, began to complain of an earache in the right ear on May 7, 1937, and the next day was taken to the Naval Hospital Dispensary where myringotomy of the right eardrum was done by Dr. J. H. Hooker. The ear drained copiously. Unfortunately, no smear or culture is available of the initial pus.

Fig. 1.—Streptococci in spinal fluid smear, May 31, 1937.

The patient is the youngest of four living children. The mother and father are living and well. Her past history was entirely negative, except for chickenpox in 1931, pertussis and German measles in 1934. No sequelae of either had been noted. She had never had any ear trouble prior to this attack.

Four days after the onset (May 11), x-ray of the mastoid was reported: "The films are not very clear, but the cells on the right side, superiorly, appear slightly blurred." From May 10 to May 20, she received a small series of x-ray treatments: to the right ear area, 282 r. units in three days, 200 r. units to the right mastoid area on the next two days, and 70 r. units to the pharynx on the fifth day. This treatment was apparently without beneficial effect.

She was first seen by the author on May 22, because it was thought that she was developing measles which was an epidemic in the community at the time.

Examination.—The child was well developed and nourished, mentally clear, alert, quiet, and cooperative. There was moderate coryza and a slight maculopapular rash on the neck and upper extremities and Koplik spots on the nasopharyngeal mucosa. There was purulent drainage from the right ear canal, and the site of the myringotomy was patent. The temperature by mouth was 104°. There was no evidence of meningeal irritation, mastoid tender-

ness or smoothness, headache, and she appeared not acutely ill. The examination was otherwise not remarkable. No reflex changes were noted at the initial examination.

Subsequent Course.—Forty-eight hours later, however, she had developed evidence of meningitis. The lids drooped bilaterally, her temperature had risen to 106.8° by rectum, and there was marked irritability, tonicity, and slight stiffness of the neck without rigidity. The eruption of measles was widespread. The knee jerks were hyperactive, Kernig's and Babinski's signs were positive on both sides, and an inexhaustible ankle and patella clonus was elicited. She complained of mild headache.

She was admitted to Alexandria Hospital at midnight on May 25. At this time she was slightly irrational, disoriented, and beginning to grow stuporous. The neck had become progressively more rigid. Lumbar puncture was performed immediately on admission. There was some increase in the pressure, and the fluid was faintly opalescent. At this time 10 c.c. of antimeningococcic serum was given intraspinally, and the fluid was sent to the laboratory for study.

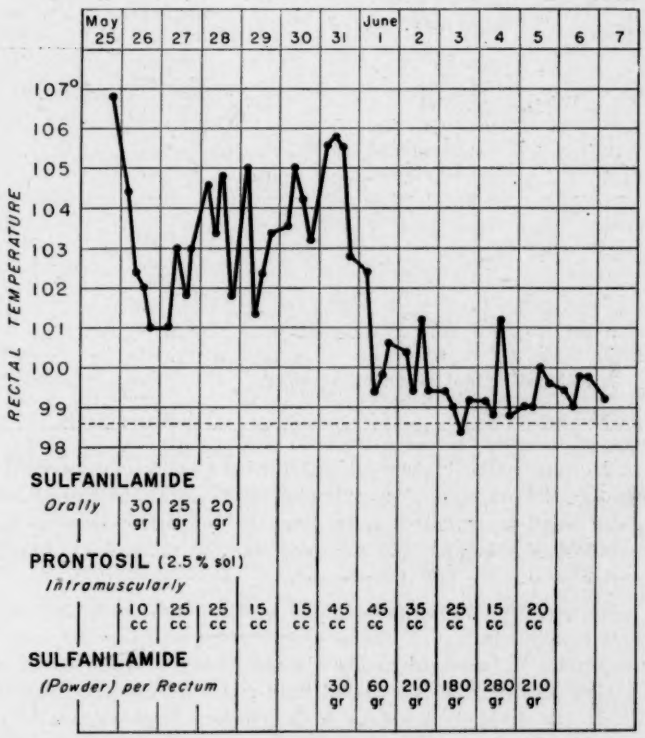

Fig. 2.—Temperature curve and doses of sulfanilamide in case of nonhemolytic streptococcic menir.gitis.

Examination of the smear showed numerous short and long chains of gram-negative streptococci (Fig. 1). As soon as the diagnosis of streptococcic meningitis was made, prontosil was begun intramuscularly and sulfanilamide tablets were given by mouth (Table II). Because of vomiting, however, the method of administration was changed after three days to a solution of powder by rectum. None of this was expelled. The qualitative test of the spinal fluid for sulfanilamide was first reported as positive on June 1, and on June 4, the concentration in the spinal fluid had reached 0.35 mg. per cubic centimeter of fluid. The following day this concentration was reported to be 0.3 mg. per cubic centimeter and decreased to 0.125 mg. two days later, in spite of the large dosage given.*

*Mr. P. T. Rees, Chief Pharmacist, U. S. Navy, did both the qualitative and quantitative analyses on the spinal fluid.

On May 26, the patient was seen in consultation with Dr. G. Haven Mankin for an opinion on the ear condition and the mastoiditis. His report: "The patient has a small amount of mucopurulent drainage from the right ear and definite tenderness over the right mastoid antrum, marked stiffness of the neck, positive Kernig. Glands at angle of jaw enlarged, throat red, veins in fundi full. Advise treatment of meningitis without operative interference R. mastoid. Suggest hot packs and irrigations R. ear every two hours."

Laboratory Data.—On admission to the hospital the leucocyte count was 19,700 with 86 per cent neutrophiles, 12 per cent lymphocytes, 2 per cent large monocytes—juvenile forms 4 per cent—stab 40 per cent. Urinalyses were entirely negative. Blood culture taken two days after admission never showed any growth. The R.B.C. on admission 4,100,000, and hemoglobin 78 per cent (Dare). The patient's blood was Type 2. The stained spinal fluid smear showed innumerable chains of streptococci (Fig. 1). The day following admission the W.B.C. were 16,650 with 92 per cent neutrophiles.

TABLE II

DATE	SULFANILAMIDE	PRONTOSIL INTRAMUSCULARLY	SULFANILAMIDE, POWDERED RECTALLY
5/26	30 gr. P.O.	10 c.c.	
5/27	25 gr. P.O.	25 c.c.	
5/28	20 gr. P.O.	25 c.c.	
5/29	None (vomiting)	15 c.c.	
5/30	None (vomiting)	15 c.c.	
5/31	None (vomiting)	45 c.c.	
	Total 75 gr.		
5/31		40 c.c.	30 gr.
6/ 1		45 c.c.	60 gr.
6/ 2		35 c.c.	210 gr.
6/ 3		25 c.c.	180 gr.
6/ 4		15 c.c.	280 gr.
6/ 5		20 c.c.	210 gr.
	Sulfanilamide treatment stopped.		Total 970 gr.
Total dosage 1,164 gr.			

Progress in Hospital.—Daily cisternal and lumbar punctures were done throughout her stay of three weeks in the hospital. Approximately 20 to 40 c.c. of cloudy fluid were removed at each tap. Four small transfusions were given as supportive treatment as follows: on May 26, 200 c.c. of blood, May 28, 200 c.c., May 31, 250 c.c. and on June 2, 250 c.c. By May 31, the eruption of measles had disappeared.

On June 1, the condition of the patient was most critical. Despite withdrawal of spinal fluid and adequate administration of fluids by vein, the situation became desperate. The streptococci were present in increased number in the smears. The cell count was increasing, the culture remaining positive, and the spinal fluid sugar decreasing. The prognosis seemed extremely grave. It was decided, therefore, to introduce a large amount of sulfanilamide by rectum since the patient was not retaining any by mouth. In divided dosage 30 gr. were given dissolved in 2 oz. warm physiologic saline solution. This was increased to 60 gr. in the next twenty-four hours, to 210 gr. the next day, to 180 gr. on the fourth, and to 280 gr. on the fifth day. Thereafter only one further dose was given; 210 gr. on the sixth day of the rectally administered drug (Table II). On the fourth day of this treatment, the smear and culture were negative but were reported positive again on the fifth day. The sixth day and thereafter the spinal fluid smear showed no streptococci, and the culture was reported as showing no growth. None of the drug was expelled from the rectum, and the ease of administration and rapidity of solution attracts itself as a splendid method of giving the drug, especially to children and to patients who are vomiting. Clinical improvement was noticed after the fifth day of the rectal administration of sulfanilamide.

Development of Toxic Symptoms.—These were noticed fairly early during the drug therapy, the earliest and most striking was the now familiar dusky violaceous cyanosis, which was especially noticeable in the lips, finger and toe tips, helices of the ears, etc. As was to

be expected when the dosage was increased, other toxic symptoms became apparent—increased irritability and uneasiness, rather marked weakness, and increased respiratory and cardiac rates. It seems now quite apparent that this overdosage caused most of the latter, because the toxic symptoms quickly subsided when the drug was withdrawn. Although sulfanilamide is apparently quickly excreted by the renal route, it is assumed that with any kidney damage this would not take place, and the resultant cumulative action of this toxic medicament might cause depression and failure of respiratory and cardiac centers. In spite of the enormous dosage the patient rallied rapidly, and, except for continuation of a transient weakness several days after withdrawal, no harmful effects were noted. When streptococci finally disappeared from the spinal fluid, her improvement was rapid; and three weeks after admission to the hospital the patient was discharged to her home.

Blood Examination on the Day of Discharge from Hospital.—Hemoglobin, 10 gm., or 87 per cent (H-H); R.B.C., 3,920,000; W.B.C., 5,350. Differential polymorphonuclears, 76 per cent; lymphocytes, 21 per cent; large monocytes, 3 per cent; R.B.C. showed slight anisocytosis. It is apparent that the drug acts directly on the invading organism. It did not cause leucocytosis and apparently did not severely depress the bone marrow.

CONCLUSIONS

A case of nonhemolytic streptococcic meningitis is reported. Treatment included prontosil and sulfanilamide in large doses, aggregating 1,164 gr. Some symptoms of toxicity are reported. Recovery was uneventful and without sequelae.

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A CONSIDERATION OF QUANTITATIVE RELATIONS BETWEEN ERYTHROCYTES, LEUCOCYTES, AND HEMOGLOBIN OF THE BLOOD*

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ALTHOUGH the origin and function of the red blood cells, the white blood cells, and the hemoglobin of the blood are quite different, each constituent gives an index of the status of the tissues from which it is derived, and in case of pathologic disturbance within the organism, one or all three may reflect alterations in the fluid matrix and other tissues of the body. In the development of these important components of the blood from their original source, there are within the body certain determining, reacting, and participating factors to which all are subjected, and in addition they are exposed to the same environmental influences within the blood stream. It would seem, therefore, that there might exist some quantitative relationships of significance among the three, since they are present in one and the same circulating tissue medium and are intimately intermingled and associated together. Yet the literature seems to contain no record of a mathematical study having been made of the blood of large numbers of normal individuals. Pursuant to a study of the complete blood picture and of the structural and functional changes characteristic of infancy,[1] as well as during later growth and development, an investigation as to the relative behavior of some of the measurements of these blood constituents has been made.

The data discussed herein were selected from some growth studies conducted in the Research Laboratory of the Children's Fund of Michigan, where monthly blood observations were made as a part of the detailed medical examination given a large group of healthy infants during their first year of life, the details of which can be found elsewhere.[2-6] There were 1,644 instances in which simultaneous observations were made of erythrocytes, leucocytes, and hemoglobin at the same clinic visit.

The methods for determining the number of erythrocytes, leucocytes, and the amount of hemoglobin in the blood were carefully standardized, and the bloods were taken at the same time of day, between the hours of 10:00 A.M. and noon. A free blood flow was established from the large toe of each subject by a Bard-Parker knife, number 11. The same Trenner hemoglobin and red blood cell pipettes, certified by the United States Bureau of Standards, were used throughout the study. The blood for the hemoglobin determination was made up

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to a 1:10 dilution in the 10 c. mm. diluting pipette with n/10 hydrochloric acid. The pipette was shaken immediately for two or three seconds and allowed to stand thirty minutes at room temperature for the color reaction to take place in the pipette bulb. The hemoglobin was then determined in the Haden-Hausser hemoglobinometer[7] which was checked at regular intervals against the Van Slyke gasometric method for estimating hemoglobin.[8] All observations were made by only three laboratory workers (B. M., M. L. S., and L. E.) to reduce the personal error factor.

Hayem's diluting solution was used for making the erythrocyte counts. A mechanical rotary angular shaker was used for agitating the hemacytometer pipettes,[6] and after mixing for ten minutes a standard distribution of cells was obtained. Only one Levy-Hausser counting chamber with the improved Neubauer ruling $\frac{1}{400}$ sq. mm. $\frac{1}{10}$ mm. deep, which had been certified by the United States Bureau of Standards, was used in this study. Especial care was taken to keep the counting chamber scrupulously clean, to discard three or four drops from the blood pipette before filling the counting chamber, to fill the chamber as carefully and quickly as possible, and to allow two or three minutes for the settling of the cells before making the red cell count. All the spaces beneath the five double rulings were counted, thus making 5 sq. mm. in all. The sum of the erythrocytes in these squares was multiplied by 10,000 to give the number of cells per cubic millimeter.

The white cell pipettes were also of the Trenner automatic type and certified by the United States Bureau of Standards. Two per cent acetic acid was used as the diluent, and the pipettes were agitated in the shaker. The five large squares of the counting chamber, including the center, were counted, making eighty small squares in all, and the sum of the cells in these squares was multiplied by forty to give the number of cells per cubic millimeter.

Since the three constituents were not always determined at each presentation of the child for blood samples, the data thus selected present an interesting problem—one entirely incidental to this investigation—in the reliability of results obtained from a small random sample of data as compared to that of the much larger number of measurements from which the sample is chosen. This is shown by the fact that the 3,541 measurements originally studied showed an average of 11.34 gm. of hemoglobin per 100 c.c. of blood, with a standard deviation of 1.25, whereas the sample of 1,644 determinations selected for this study averaged 11.40 gm. of hemoglobin per 100 c.c. of blood, with a standard deviation of 1.38. That the results for this smaller sample are well within the limits of expected variation is obvious without application of further numerical proof.

The data from 1,644 simultaneous determinations of hemoglobin, erythrocytes, and leucocytes on 364 well infants are presented in three so-called correlation tables, erythrocyte-hemoglobin, hemoglobin-leucocyte, and erythrocyte-leucocyte, Tables I, II, and III, respectively. In case of the hemoglobin, the frequency classes are designated by the midvalue of the class interval, stated in grams per 100 c.c. of blood; the frequency classes of the red blood cells are designated by the lower limit of the group, stated in units of 10,000 per c. mm.; and the white blood cells are measured in units of 100 per c. mm., with the lower limit of the class interval being given. It should be noted that the length of the class

TABLE

HEMOGLOBIN-ERYTHROCYTE
(1644 BLOOD

HEMOGLOBIN IN GRAMS PER 100 C.C. OF BLOOD	ERYTHROCYTES IN UNITS																				
	178	240	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440
6.5	1	1																			
7.0		1																			
7.5		1																		1	
8.0									1	1		1	1	2							
8.5						1			1										1		1
9.0						1		1	2	2	1	2	1		1	1	2	1	2	1	
9.5		1		1		1	2	2	1	2	2	1	2	2	3	3	1	3	5	7	1
10.0		1	1	1	3		1	3	5	9	9	14	13	10	15	11	8	20	10	12	10
11.0					1	1	1	6	12	13	12	9	19	28	22	32	35	32	43	44	34
12.0					1	1			4	3	8	10	11	9	18	21	25	26	34	36	40
13.0								2	1	1	1	5	4	2	3	3	12	16	13	11	6
14.0														1	3	3	4	5	2	1	3
15.0												1					1		2		1
16.0																		1	1	1	1
17.0																					
18.0																					
fx	1	5	1	2	5	5	4	14	27	31	33	43	51	54	65	74	88	104	113	114	97

Hemoglobin = y
$M_y = 11.40$
$\sigma_y = 1.38$

Erythrocyte = x
$M_x = 440.20$
$\sigma_x = 63.34$

$r_{xy} = 0.26$
$PE_{r_{xy}} = 0.02$
$\sigma\epsilon = \sigma_y \sqrt{1-r^2_{xy}} = 1.33$
$\frac{\sigma_y}{\sigma_x} . r_{xy} = 0.01$

TABLE

HEMOGLOBIN-LEUCOCYTE
(1644 BLOOD

HEMOGLOBIN IN GRAMS PER 100 C.C. OF BLOOD	LEUCOCYTES IN UNITS																	
	23	40	46	52	58	64	70	76	82	88	94	100	106	112	118	124	130
6.5															1		1
7.0														1	1		
7.5					1				3				1				1
8.0								2	1	1	1		5				2
8.5								5			2		1				
9.0					2	1		5	2	3	3	2	2		2		
9.5			1	3	2	6	5	9	5	3	9	3	4	4	4	3	4
10.0		1	2	3	9	11	13	14	24	20	15	18	16	18	21	2	8
11.0	1		4	7	16	23	33	46	52	42	52	54	41	39	45	29	22
12.0			4	8	13	13	25	30	40	33	43	44	39	36	34	24	18
13.0		2	1	1	4	4	8	7	16	14	7	16	11	10	15	10	6
14.0			1	1	1	4	2	4	6	3	3	6	5	6	3	2	3
15.0				1	1	1		1	1	2	1	3	2	2		1	
16.0							1			2	2	1	1	1			2
17.0						1	1						1		1		
18.0									1						1		1
fz	1	3	13	24	49	64	88	123	151	123	138	147	129	117	128	71	68

Hemoglobin=y
$M_y = 11.40$
$\sigma_y = 1.38$

Leucocyte=z
$M_z = 104.20$
$\sigma_z = 29.40$

$r_{yz} = 0.07$
$PE_{r_{yz}} = 0.02$
$\sigma\epsilon = \sigma_y \sqrt{1-r^2_{yz}} = 1.38$
$\frac{\sigma_y}{\sigma_z} . r_{yz} = 0.00$

I

DETERMINATIONS OF 364 INFANTS
DETERMINATIONS)

OF 10,000 PER C.MM.																							
450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	670	680		*fy*
																							2
							1																2
		1		1			1								1								6
				3				1	1	1													12
				3	1				1						1								10
1		3	1			1	1																25
4	4	2	5	5	3		6	3		1													73
7	15	14	8	5	3	4	4	1	2	1	3	1	1	1									226
34	36	29	25	23	19	21	11	4	10	2	4	2	3			1							568
38	35	25	20	32	17	18	11	5	7	4	2	3	1	4	1								470
9	7	10	10	10	3	2	2	4	2	1	2	3		1	1	1			1		2		151
1	2	4	5	5	2	1	3	3	1	2		1		2					1				55
1	1	1	1	3	3	2			2	2	1	1											23
1					1		2	1	1	1	1	1											13
	2						1			1										1			5
										1							1	1					3
96	102	89	75	90	52	49	43	22	27	17	13	12	5	8	4	2	1	1	2	1	2		1644

II

DETERMINATIONS OF 364 INFANTS
DETERMINATIONS)

OF 100 PER C.MM.																			
136	142	148	154	160	166	172	178	184	190	196	202	208	220	226	268	298			*fy*
																			2
																			2
																			6
																			12
	1				1														10
	1	2																	25
3	2		1			1				1									73
7	5	7	4	1	1	3	1			1			1						226
9	14	6	7	5	7	4	2	1	1	1	1	1		1	1	1			568
16	7	13	3	10	4	2	3		1	4		1		2					470
2	5	2	2	1	1	5					1								151
2	2	1																	55
2			1	3	1														23
1	1				1														13
					1														5
																			3
42	38	31	18	20	17	15	6	1	2	7	2	2	1	3	1	1			1644

TABLE

LEUCOCYTE-ERYTHROCYTE

LEUCOCYTES IN UNITS OF 100 PER C.MM.	ERYTHROCYTES IN UNITS																				
	178	240	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440
23																					
40																				1	
46											1				1	1	3				2
52								1			1		2	1	1		2	5	1	3	
58					1		1	2	1	1		3	2	1	2	4	3	2	2	2	5
64				1				1	2	4		3	5		3	1	9	1	5	3	7
70		1							2	2	3		6	3	5	5	2	6	4	8	10
76				1	1	1	1			2	7	6	2	5	8	4	6	3	12	11	4
82		1				1		1	4	1		5	3	5	8	10	5	11	12	13	10
88		1				1		2	2	6	3	2	4	4	6	1	9	6	8	7	5
94					2	1	1	2	7	3	5	4	4	7	6	5	6	6	12	10	4
100								1	2	2	2	3	3	6	3	10	10	9	5	12	7
106					1				2	4	1	3	4	7	5	7	8	8	7	6	7
112		1	1			1	1			2	1	2	3	3	7	7	7	8	10	8	4
118	1							1	3	1	4	4	3	3	2	5	5	14	8	10	9
124										1	2	2	1	1	1	5	3	7	5	3	5
130		1							1		2	1	2	2		3	2	9	7	5	4
136										2		1	1	1	1			4	5		4
142								1			1	1	2	1	1	3	1	1	3	3	3
148												1	2		4	1	2			2	3
154								1						1		1				1	2
160													1				2		2	2	
166												2		1		1	1		1	1	1
172								1	1					1	1		1	3	1		1
178														1			1		1	1	
184																					
190																			1		
196													1					1		1	
202																					
208																			1		
220																					
226																				1	
268																					
298																					
fx	1	5	1	2	5	5	4	14	27	31	33	43	51	54	65	74	88	104	113	114	97

Erythrocyte = x
$M_x = 440.20$
$\sigma_x = 63.34$

Leucocyte = z
$M_z = 104.20$
$\sigma_z = 29.40$

$r_{xz} = 0.13$
$P E_{r_{xz}} = 0.02$
$\sigma_\epsilon = \sigma_z \sqrt{1-r^2_{xz}} = 29.14$
$\frac{\sigma_z}{\sigma_x} . r_{xz} = 0.06$

interval in the hemoglobin classification is not uniform throughout, the length in the lower half of the table being twice that of the upper half. If we designate the measure of the red blood cells by the letter x, the measure of the hemoglobin by the letter y, and the measure of the white blood cells by the letter z, then the pictures presented by the tables tell us that there are evidently no clearly defined functional relationships of the form $y = f(x)$, or $y = F(x, z)$. The widely used measure of linear relationship between two variable quantities, namely, the Pearson correlation coefficient, is evidently small though from the graphical standpoint, a linear relation in each of the three cases seems to be the most

III

DETERMINATIONS OF 364 INFANTS

OF 10,000 PER C.MM.																									
450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	670	680	fz
									1													1
								1			1											3
1	2			1			1															13
2			2	1				1		1												24
2	3	2	1	2	1	2	3			1												49
2	5	2	3	1	2	2	1				1											64
2	4	6	4	2	2	2	3	1	1	1				1					1	1		88
10	8	5	4	14	2	2	2		1	1												123
11	12	15	5	3	5	4	1		2	1			1	1								151
14	8	4	9	4	2	2	6		2		1	3			1							123
8	3	5	6	10	3	4	3	2	6	1	1					1						138
8	13	11	9	8	2	2	6	5		2	2	1		3								147
7	8	6	6	9	5	4		3	3	3	1	2									2	129
5	6	5	5	5	4	7	2	2	6		2		1	1								117
4	7	10	6	13	4	3	3	1		1	1		1				1					128
5	4		5	6	6	1	2	2	1	1		1				1						71
3	2	4	2	4	4	3	1		1	2			1		1			1				68
4	3	3			3	1	4	1		1	1	1							1			42
		3	2	2	3	2	1		1					1	2							38
2	5	4		1		1	1				1			1								31
2	1		1		1	1		1	1	1	1	2										18
3	3	1	1	1	1		1	1				1										20
	2	1		1	2	2	1															17
			2			1			1				1									15
						2																6
								1														1
				1																		2
2			1			1																7
			1				1															2
	1																					2
	1																					1
		1										1										3
	1																					1
				1																		1
96	102	89	75	90	52	49	43	22	27	17	13	12	5	8	4	2	1	1	2	1	2	1,644

logical one to assume. For the data of each of the tables the following constants have consequently been calculated for the two variables, say α, β, of the respective tables: (a) the correlation coefficient, $r_{\alpha\beta}$, (b) the probable error of the correlation coefficient, P.E. $r_{\alpha\beta}$, (c) the slope, $m = \frac{\sigma\alpha}{\sigma\beta}.r_{\alpha\beta}$, of the line of best fit to the data by the method of least squares (remembering that this line always passes through the means of the two distributions), and (d), the standard error of prediction, $\sigma\varepsilon$, of values of α from β by this line of best fit.

Of the three constituents under consideration, probably the most often associated in discussion are the red blood cells and the hemoglobin. From Table I, which represents the data of these measurements, we find $r_{xy} = 0.26$, P.E.$_{r_{xy}} = 0.02$, $\frac{\sigma y}{\sigma x} r_{xy} = 0.01$, and $\sigma\varepsilon = 1.33$. Authors vary in their opinions as to the size of a correlation coefficient necessary to be considered important; however, when we consider that the standard deviation of the observed y's (hemoglobin) is 1.38 and that of the y's predicted by aid of the associated x's (red blood cells) is 1.33, the value of information regarding the erythrocyte count of an infant is essentially valueless as an aid in making any statement as to what the hemoglobin content of his blood should be. The fact that r_{xy} (and consequently m) is positive, although small, does say that an increase in the red blood cell count is usually accompanied by a slight increase in the amount of hemoglobin.

A geometrical interpretation of the hemoglobin-leucocyte correlation, Table II, would seem to tell a very similar story to that of Table I. These data presented in a table in which the frequency class interval for the white blood cell counts is taken as three units (which was originally done with essentially the same results, but offered difficulties in presentation) instead of six as in this table, makes the independence of these two sets of measurements much more in evidence. For these data we find $r_{yz} = 0.07$, P.E.$_{r_{yz}} = 0.02$, $\frac{\sigma y}{\sigma z} . r_{yz} = 0.00$, and $\sigma\varepsilon = 1.38$. The constants show the distinct lack of any linear dependence of the amount of hemoglobin upon the number of white blood cells.

The scatter of the entires in Table III also pictures a lack of quantitative dependence of the two types of blood cells measured by this table, namely, the red and the white blood cells. Graphically this table also makes a clearer presentation when the leucocyte class interval is taken as three instead of six, but the numerical value of the statistical constants indicates sufficient directness. Here $r_{xz} = 0.13$, P.E.$_{r_{xz}} = 0.02$, $\frac{\sigma_z}{\sigma_x} r_{xz} = 0.06$, $r_{xz} = 0.06$, and $\sigma\varepsilon = 29.14$. From so large a number of measurements as we have here, 1,644, upon which to base our conclusions, the slight increase in the leucocyte count accompanying a given increase in the erythrocyte count is negligible in value for predictive purposes. With $\sigma_z = 29.40$ and $\sigma\varepsilon = 29.14$ any information gained from a knowledge of the number of red blood cells of an infant offers us no help in estimating his white blood cell count.

If we evaluate the multiple correlation coefficient on the assumption of y being expressible as a linear function of both x and z, say $y = f(x, z)$, we find that $r_{xyz} = 0.27$. The standard error of the y's (hemoglobin values) as predicted from the corresponding x's and z's is found to be 1.33. So again, remembering that $\sigma_y = 1.38$, we are in no better position to estimate the value of the hemoglobin of a given individual knowing both his red blood cell and white blood cell counts than if we had no information concerning them. A similar statement can be made about any one of the three quantities with respect to the other two.

As an indication of whether or not the results set forth in this paper may be said to apply equally well to persons at all ages, it may be stated that a separate study of 206 blood determinations from 11 adult women does corroborate these results in every essential detail.

DISCUSSION

In an investigation involving two or more variable constituents, each quantitatively measurable and closely associated, it is important to have a measure of that association, and it is equally desirable to definitely establish their independence if it exists. The variables considered in this study are the three important blood constituents, hemoglobin, red blood cells, and white blood cells. Correlation tables have been prepared for erythrocyte-hemoglobin, hemoglobin-leucocyte, and erythrocyte-leucocyte relationships. It is apparent from these correlations that the levels of hemoglobin, red and white cells are quite independent of each other within certain limits, and from the standpoint of clinical application, this statistical fact verifies the generally accepted precept that routine determination of any one of these components of the blood will not give any indication of the exact level of the other two elements. This fact is applicable to well infants, and not to pathologic states, since it is based on data collected from well infants under carefully supervised feeding management.

SUMMARY

1. On 364 well infants during their initial twelve months of life when they were kept on a satisfactory and carefully supervised dietary regimen, 1,644 simultaneous determinations of hemoglobin, red blood cell, and white blood cell counts have been made. Detailed anthropometric measurements and monthly medical examinations demonstrated that on the average the infants were growing as well as or better than, the available standards.

2. Statistical evidence from correlation tables for erythrocyte-hemoglobin, hemoglobin-leucocyte, and erythrocyte-leucocyte blood components are presented, which establishes mathematically the precept that quantitatively the hemoglobin, the red blood cell, and the white blood cell counts are independent of one another within certain limits. Although a rise in erythrocytes is usually accompanied by a slight rise in hemoglobin, a knowledge of the red cell count is of little value in estimating the actual hemoglobin level. Similarly, if one knows the hemoglobin value, it is not possible to predict what the red cell count will be, nor will a white cell count be of any value in gauging the number of red blood cells, or vice versa.

3. This paper also brings out the important fact that the hemoglobin, erythrocytes, and leucocytes are independent of each other in the blood of normal adult women.

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FACTORS INFLUENCING THE PRODUCTION OF GUINEA PIG COMPLEMENT OF SATISFACTORY TITER*

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WHILE guinea pig serum is the most important source of complement for Wassermann and other complement fixation reactions, there is still considerable disagreement concerning the influence of certain factors upon its hemolytic activity. Such conflicting reports, together with personal observations on complement deficiency, made it desirable to study some of these conditions, including such factors concerning the guinea pig itself, as age, weight, sex, litter-mate differences, and the effect of feeding, sunlight, and temperature.

Rich[1] reported a strain of complement-deficient guinea pigs at the Vermont Agricultural Experiment Station. Genetic studies indicated that complement was not a universal blood factor and could be controlled by selective mating in conformity with Mendel's law. Hyde,[2] after ten years' work with this same complement-deficient strain, believed that lack of complement was a character inherited as a simple recessive Mendelian unit. Ruediger[3] was able to increase the titer of his collection of guinea pigs over a period of three years by breeding only those with good titers.

Age.—Browning[4] stated that the complement content of guinea pigs tended to be lowest immediately after birth but underwent a marked increase during the first three months of life. Friedberger and Gurwitz[5] reported that complement titers of newborn guinea pigs were about the same as the mother. Hyde[2] found no appreciable difference between the complement titer of young guinea pigs and mature animals, whereas Osborn[6] stated that in young rats there is a marked tendency toward low complement titer.

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Weight.—Friedberger and Gurwitz[7] found serums from 150- to 600-gm. guinea pigs showed marked differences in complement content, wholly independent of age and weight. Kolmer[8] did not find any variation in hemolytic activity between a group of 200- to 280-gm. pigs and another group of 400 to 580 gm., but did recommend the heavier animals, as serums of younger ones were more likely to be anticomplementary. Cummer[9] recommended pigs weighing 400 gm. or more, because small pigs yielded little blood and that weak in complement.

Sex.—For satisfactory complement Kolmer[8] recommended the use of large, well-nourished animals, males preferred, which had not been fed within twelve to twenty-four hours of bleeding. He also avoided using pregnant animals, despite the fact that he found little difference between their titer and males of the same weight, and suggested that pooled serums of three or more pigs should be used. Cummer[9] advised that pregnant animals be avoided and pooled serums of three or more pigs be used, since there is considerable variation between different pigs. Wadsworth[10] stated that three, and preferably six or more pooled serums are desirable, and to avoid chylous serum advised not bleeding until several hours after feeding. Simmons[11] suggested that blood for complement be obtained from three or more full-grown, healthy male guinea pigs. Although giving no reason, he stated that females should be avoided because of the possibility of pregnancy. A deficiency in complement was associated with pregnancy according to Browning.[4] Rich[1] found that neither castration nor pregnancy affected the complement content of guinea pigs. Gradwohl[12] merely stated that complement varied from time to time in animals and was present in particularly large amounts in guinea pigs. Wadsworth, Harris, and Gilbert[13] stated that healthy animals, preferably males, should be tested for hemolytic activity and fixability, and pooled serums of three or more satisfactory animals should be used. The same statement also appeared in a report by Gilbert.[14]

Feed.—Simola and Brunius[15] reported that neither A nor C avitaminosis nor the administration of vitamin D in the form of irradiated ergosterol to guinea pigs had any effect on complement. With rats on a diet deficient in vitamin A, Osborn[6] observed lower titers than with controls receiving cod liver oil. While not bearing directly on complement, Madsen, McCay, and Maynard[16] found it difficult to raise guinea pigs using a synthetic diet free from hay and grain but including cod liver oil or vitamin A-D concentrate. Hilgers and Zain[17] stated that acid and alkali diets generally have no effect on complement titer.

A report of the United States Department of Agriculture[18] recommended that guinea pigs be given water, salt, dry grains, hay, and a daily feed of green stuffs. Rich,[1] using many hundreds of guinea pigs and feeding a variety of foods over a period of years, could find little evidence that diet had any effect on complement. Kolmer,[8] although admitting the lack of systematized and scientifically regulated diet experiments, stated that a mixed diet maintaining animals in good health and normal weight was sufficient to yield acceptable complement. In order to prevent dilution of blood by hydremia, Cummer[9] recommended that pigs have a diet of oats only for at least a day before bleeding. Austin,[19] working on the nonspecific fixability of guinea pig serum with Bordet's antigen, kept animals on a diet of oats, cabbage, and alfalfa, fed dry in winter and green in summer. Browning[4] stated, based on the work of Griffith and Scott, that in guinea pigs

TABLE I

A COMPARISON OF COMPLEMENT TITERS, AGE, AND WEIGHT OF GUINEA PIGS HOUSED OUTDOORS AND INDOORS ON DIFFERENT RATIONS

(Results of titration expressed as c.c. of 1:10 complement required to hemolyze 0.5 c.c. of sheep cells with 2 units of hemolysin [0.5 c.c. of 1:2,000 dilution])

CAGE		GUINEA PIG				AGE, TITER, AND WEIGHT												
						1935								1936				
NUMBER AND LOCATION	FEED	NUMBER	LITTER NUMBER	SEX	WEIGHT 6/4	6/14	6/29	7/30	8/31	10/2	11/1	12/3	12/31	1/30	2/29	3/28	5/2	6/6
1 Outside	Wheat Oats	409	1	M	90	0.18 95	0.24 120	0.22 231	0.18 356	0.22 459	0.18 532	0.10 545	0.10 535	0.12 576	0.10 622	0.08 660	0.12 653	0.14 641†
	Greens	198	9	M	274	0.18 272	0.18 302	0.22 428	0.24 554	0.20 673	0.16 762	0.10 772	0.10 775	0.10 862	0.08 947	0.08 930	0.10 1002	0.10 1013
		408	10	M	90				0.26+ 124	0.26+ 218	0.20 312	0.18 382	0.10* 445	0.12 510	0.10 559	0.12 628	0.10 636	0.10 615
		410	3	F	104	0.26+ 105	– 122	0.22 232	0.16 376	0.16 502	0.20 637	0.14 611	0.16 654	0.24 684	0.18 704	0.26 763	0.26+ 1004	0.26 710
		187	4	F	142	0.16 144	0.16 164	0.20 259	0.20 355	0.22 428	0.18 506	0.22 628	0.12 494	0.22 533	0.12 633	0.26 706	0.18 598	0.24 646
1 Inside	Wheat Oats	177	1	M	92	0.22 104	0.24 120	0.22 244	0.20 370	0.16* 457								
	Greens	194	7	M	136	0.22 160	0.20 198	0.22 336	0.22 448	0.24 526	0.16 600	0.16* 626						
		301	12	M	81				0.26+ 120	0.26+ 200	0.18 289	0.10 376	0.08* 463					
		406	4	F	190	0.16 203	0.18 236	0.16 368	0.18 533	0.26+ 637	0.20 558	0.20 595	0.26 718	0.20 846	0.14 937	0.12 1024	0.10 1062	0.20 1101
		191	6	F	86	0.24 98	0.24 119	0.22 260	0.20 418	0.22 553	0.14 468	0.12 535	0.16 726	0.26+ 639	0.12 636	0.12 710	0.18 764	0.14 825
2 Outside	Pellets Oats	418	2	M	95	0.20 123	0.20 184	0.18 327	0.20 470	0.20 556	0.18 608	0.10 638	0.10 624	0.08 612	0.10 658	0.10 713	0.12 715	0.14 668

*Died.

†Weight expressed in grams.

NOTE: Large increases in weight attended by subsequent fall in weight in females was due to pregnancy.

TABLE I—CONT'D

	Cabbage	413	8	M	107	0.22 140	0.22 186	0.20 321	0.20 468	0.12 541	0.16 680	0.14 642	0.10 697	0.14 623	0.12 742	0.12 777	0.12 812	0.10 859
		178	1	F	78	0.26+ 109	0.26+ 156	0.18 282	0.24 439	0.12 513	0.16 634	0.16 685	0.20 808	0.12 580	0.26 860	0.24 632	0.20 717	0.26+ 866
		403	5	F	206	0.20 254	0.16 300	0.20 442	0.20 572	0.20 644	0.12 745	0.12 828	0.22 1064	0.10 761	0.20 960	0.22 802	0.24 927	0.26 1200
2 Inside	Pellets Oats	186	4	M	135	0.20* 167												
	Cabbage	189	5	M	180	0.24 222	0.20 302	0.18 492	0.18 622	0.22 733	0.16 818	0.16 813	0.22 824	0.10 854	0.10 910	0.08 958	0.08 961	0.12 1017
		195	7	M	126	0.20 167	0.24 226	0.20 402	0.24 555	0.24 675	0.16 774	0.16 744	0.14 758	0.10 752	0.10 838	0.10 891	0.14 887	0.16 943
		420	12	M	120				0.24 166	0.20 372	0.16 502	0.14 526	0.12 564	0.12 639	0.12 701	0.10 732	0.12 740	0.10 825
		180	2	F	85	0.22 126	0.20 198	0.12 344	0.14 512	0.14 677	0.24 922	0.12* 668						
3 Outside	Ready-mixed feed	401	6	M	80	0.26+ 108	0.24 131	0.24 256	0.22 421	0.20 500	0.10 602	0.10 620	0.08 598	0.08 604	0.10 701	0.10 663	0.08 643	0.12 738
	Cabbage	316	11	M	73				0.20 160	0.16 336	0.14 468	0.10 540	0.08 530	0.08 550	0.08 655	0.08 650	0.06 627	0.06 649
		183	3	F	78	– 94	0.24 113	0.22 230	0.24 406	0.24 520	0.18 625	0.20 684	0.20 809	0.20 616	0.24 980	0.22 746	0.26+ 1010	0.12 753
		422	8	F	92	0.26+ 122	0.24 165	0.12 287	0.14 436	0.12 525	0.16 631	0.26 796	0.12 570	0.22 602	0.22 877	0.12 714	0.24 884	0.20 796
		181	2	F	102	0.26+* 121												
3 Inside	Ready-mixed feed	415	6	M	77	0.24 114	0.20 167	0.10 358	0.10 520	0.12 834	0.14 684	0.10 676	0.10 698	0.08 725	0.10 778	0.10 796	0.10 800	0.08 850
	Cabbage	184	3	F	136	0.24 184	0.20 254	0.22 396	0.24 504	0.24 745	0.26+ 656	0.18 680	0.18 815	0.18 768	0.20 977	0.22 1382	0.24 856	0.26 800
		190	5	F	193	0.26+ 247	0.26+ 300	0.12 415	0.22 560	0.24 822	0.24 744	0.26 881	0.26+ 712	0.22 894	0.20 936	0.22 886	0.26+ 1434	0.20 925
		199	9	F	202	*												

nutrition had no definite relation to complement. Ruediger[3] found little difference in the titer of guinea pigs fed during the winter on various mixtures of vegetables in addition to a basic diet of bread, hay, and oats.

Temperature and Sunlight.—Little information has been available on the effect of sunlight and temperature upon guinea pigs. A temperature below 65° F. was unsuitable for guinea pigs according to a report of the United States Department of Agriculture.[18] Remlinger and Bailly[20] found that exposure of rodents to the sun for only half an hour would cause death, and concluded that their susceptibility was probably due to habitual sheltered existence.

EXPERIMENTAL

Preparation of Materials.—Guinea pigs were lightly etherized and 2 c.c. of heart's blood withdrawn for complement. This was placed in a sterile agglutination tube, slanted, allowed to stand at room temperature for one hour and then placed in a refrigerator (7° to 9° C.) for twenty-four hours before the titer was determined. Each sample was centrifuged to obtain clear serum. The same lot of hemolysin, obtained from Difco Laboratories, was used throughout the experiments, and was titered before each use.

Corpuscles were obtained from a ewe, blood from the jugular vein being aseptically aspirated into a 30-c.c. syringe, immediately expelled into a sterile flask containing glass beads, shaken for about ten minutes to defibrinate the blood, and refrigerated. The corpuscles were washed the same day, three times or more, packed by centrifuging for twenty minutes, made up to a 50 per cent suspension, refrigerated and used within two days. The corpuscles were made up to a 2 per cent suspension in saline for use in the tests. Throughout the work all dilutions were made with day-old saline only,[21] prepared from 8.5 gm. of C. P. sodium chloride, made up to 1,000 c.c. with cold tap water, and heated in flowing steam for thirty minutes.

Complement Titration.—The complement was titrated by determining the least amount necessary to bring about complete hemolysis of 0.5 c.c. of a 2 per cent suspension of sheep cells in the presence of two units of hemolysin contained in 0.5 c.c. The complement was diluted 1:10 and varying amounts were used, ranging from 0.06 to 0.26 c.c., with 0.02 c.c. intervals. The total volume was made up to 3 c.c. with saline, hemolysin, and complement controls set up for each titration and titers recorded after a one-hour incubation in a water bath at 37° C. All mention made hereafter of complement titers refers to dilutions of 1:10.

Animals.—Nine different litters of guinea pigs were received on June 4, 1935, and three more litters were added later to replace animals that had died. The variations in weight as listed in Table I show they varied somewhat in age, only litters 5 and 9 apparently being over three weeks old, since pigs at birth vary from approximately 40 gm. to 90 gm.

Distribution.—The animals were distributed in various cages as shown in Table II, so comparisons could be made of litter mates reared under various conditions. Three cages were placed outdoors to determine any effect of sunlight and temperature, these remaining outside regardless of weather, except from

January 23 to February 26, 1936, when unusually heavy and continued snowfall made it necessary to move them to an unheated barn. Three cages were kept indoors in a steam-heated animal house where the temperature could be regulated throughout the year.

The animals were placed in rectangular cages 3 by 1½ by 1 foot deep, set on legs 1½ feet high. The sides of the cages were covered with fine mesh wire nailed to a wooden frame. The top, covered with the same type of wire, was hinged so that it could be raised. The floor was constructed of wood over which a heavy, removable, galvanized iron tray was placed. In the outdoor cages one-half of each, including top, sides, and corresponding end, was covered with heavy tar roofing paper to protect the animals from the weather. A small wooden box, one end of which had an opening, was placed under the covered end to give additional protection. The other half of the cage remained open for the admission of sunlight.

Rations.—Guinea pigs placed in the three outdoor and three indoor cages were maintained entirely on one of the following three rations commonly used, fresh water being available at all times. Ration 1 was a grain mixture of wheat and oats, with greens (cabbage, kale, lettuce, grass, or spinach) fed each day. Ration 2 contained oats and a pellet food consisting of wheat germ, soy bean oil meal, corn germ meal, alfalfa leaf meal, ground oats, wheat middlings, yellow corn meal, blackstrap molasses, calcium carbonate, and iodized salt. Cabbage was also fed daily as a source of vitamin C. Ration 3 was a commercially prepared, ready-mixed product, consisting of chopped alfalfa, crushed oats, yellow cracked corn, blackstrap molasses, and a pellet feed composed of wheat germ meal, soy bean oil meal, corn germ meal, wheat middlings, calcium carbonate, and iodized salt. Cabbage was fed with this ration on alternate days. The guinea pigs were fed and watered in the early morning each day, and the cages were cleaned and bedded twice a week.

RESULTS

The first complement titration was made on June 14, 1935, another two weeks later, and the animals were bled (feed being removed for eighteen to twenty-four hours before bleeding), and serums titrated monthly thereafter through June 6, 1936. Weights were recorded each week, but are shown in Table I only at the time of each titration.

Data are available on twenty-five of the twenty-eight animals used during this work. Of fifteen males four died before the completion of the experiment, no data being collected on one, and of thirteen females three died without information being obtained on two.

Sex.—In all cages, regardless of diet, the blood serum of *male* guinea pigs gradually increased in titer between the third and sixth month. Of animals surviving until completion of the test period, however, five (distributed in four different cages) showed some slight drop in titer during the last month or two (May and June), the titer of six remained about equal to the highest titer reached, while three that died before completion of the experiment showed an increased titer. As shown in Table I, the weights of these animals could not be correlated

TABLE II

A COMPARISON OF COMPLEMENT TITERS, AGE, AND SEX OF GUINEA PIG LITTER MATES

(Results of titration expressed as c.c. of 1:10 complement required to hemolyze 0.5 c.c. of sheep cells with 2 units of hemolysin [0.5 c.c. of 1:2,000 dilution])

GUINEA PIG				DATE OF DETERMINING TITER											
				1935								1936			
LITTER NUMBER	SEX	NUMBER	CAGE NUMBER AND LOCATION	6/14	6/29	7/30	8/31	10/2	11/1	12/3	12/31	1/30	2/29	3/28	5/2	6/6
1	M	409	1, outside	0.18	0.24	0.22	0.18	0.22	0.18	0.10	0.10	0.12	0.10	0.08	0.12	0.14
	M	177	1, inside	0.22	0.24	0.22	0.20	0.16*								
	F	178	2, outside	0.26+	0.26+	0.18	0.24	0.12	0.16	0.16	0.20	0.12	0.26	0.24	0.20	0.26+
2	M	418	2, outside	0.20	0.20	0.18	0.20	0.20	0.18	0.10	0.10	0.08	0.10	0.10	0.12	0.14
	F	180	2, inside	0.22	0.20	0.12	0.14	0.14	0.24	0.12*						
	F	181	3, outside	0.26+*												
3	F	410	1, outside	0.26	–	0.22	0.16	0.16	0.20	0.14	0.16	0.24	0.18	0.26	0.26+	0.26
	F	183	3, outside	–	0.24	0.22	0.24	0.24	0.18	0.20	0.20	0.20	0.24	0.22	0.26+	0.12
	F	184	3, inside	0.24	0.20	0.22	0.24	0.24	0.26+	0.18	0.18	0.18	0.20	0.22	0.24	0.26
4	M	186	2, inside	0.20*												
	F	406	1, inside	0.16	0.18	0.16	0.18	0.26+	0.20	0.20	0.26	0.20	0.14	0.12	0.10	0.20
	F	187	1, outside	0.16	0.16	0.20	0.20	0.22	0.18	0.22	0.12	0.22	0.12	0.26	0.18	0.24
5	M	189	2, inside	0.24	0.20	0.18	0.18	0.22	0.16	0.16	0.22	0.10	0.10	0.08	0.08	0.12
	F	403	2, outside	0.20	0.16	0.20	0.20	0.20	0.12	0.12	0.22	0.10	0.20	0.22	0.24	0.26
	F	190	3, inside	0.26+	0.26+	0.12	0.22	0.24	0.24	0.26	0.26+	0.22	0.20	0.22	0.26+	0.20
6	M	415	3, inside	0.24	0.20	0.10	0.10	0.12	0.14	0.10	0.10	0.08	0.10	0.10	0.10	0.08
	M	401	3, outside	0.26+	0.24	0.24	0.22	0.20	0.10	0.10	0.08	0.08	0.10	0.10	0.08	0.12
	F	191	1, inside	0.24	0.24	0.22	0.20	0.22	0.14	0.12	0.16	0.26+	0.12	0.12	0.18	0.14
7	M	194	1, inside	0.22	0.20	0.22	0.22	0.24	0.16	0.16*						
	M	195	2, inside	0.20	0.24	0.20	0.24	0.24	0.16	0.16	0.14	0.10	0.10	0.10	0.14	0.16
8	M	413	2, outside	0.22	0.22	0.20	0.20	0.12	0.16	0.14	0.10	0.14	0.12	0.12	0.12	0.10
	F	422	3, outside	0.26+	0.24	0.12	0.14	0.12	0.16	0.26	0.12	0.22	0.22	0.12	0.24	0.20
9	M	198	1, outside	0.18	0.18	0.22	0.24	0.20	0.16	0.10	0.10	0.10	0.08	0.08	0.10	0.10
	F	199	3, inside	*												
10†	M	408	1, outside				0.26+	0.26+	0.20	0.18	0.10	0.12	0.10	0.12	0.10	0.10
11†	M	316	3, outside				0.20	0.16	0.14	0.10	0.08	0.08	0.08	0.08	0.06	0.06
12†	M	420	2, inside				0.24	0.20	0.16	0.14	0.12	0.12	0.12	0.10	0.12	0.10
	M	301	1, inside				0.26+	0.26+	0.18	0.10	0.08*					

*Died.

†Litters 10, 11, and 12 were added August 31, 1935, to replace animals that died.

with the slight drop in complement strength, which agrees with the considerable individual variation often reported previously in mature animals.

The *females* almost invariably showed a considerably lower titer than males, beginning between the fourth and sixth month, although their original complement content might have been equal or better.* This usual loss of hemolytic activity was probably due to pregnancy, a factor discounted by certain investigators. An examination of Table I shows this lowering of complement content during pregnancy. Larger increases and decreases in weight, due to pregnancy and parturition, were more evident in the weekly weights recorded than in the monthly weights shown in Table I. Because females will almost invariably breed as soon as the young are delivered, after the first several months they were nearly always pregnant. The titers of the females, while usually poor during pregnancy, were lowest near the end of their terms. Frequently when (Table I) a litter had been born, as indicated by the weights, and the female had not begun to show signs of another pregnancy, the titers were considerably higher.

Because of this interference of *pregnancy* with complement titer, few, if any, conclusions could be drawn comparing litter mates or regarding the influence of feeding, sunlight, temperature, or weight on females.

Age and Weight.—Guinea pigs reach maturity in from four to five months according to a report of the United States Department of Agriculture,[18] but, as seen in Table I, the maximum weight was not reached at that time. Whenever the complement of male animals increased to 0.16 c.c., they had also reached a weight of 445 gm. or more (Table I) except three animals of different litters, housing and diet giving this titer at 336 to 376 gm. Only two (of the three above exceptions) pigs reached a titer of 0.1 c.c. before weighing 525 gm., and of all males giving this titer eight, or 57 per cent, weighed at least 600 gm.

Young male animals did not have a titer of 0.14 c.c. or better until approximately four months or older, and the majority were between five and seven months old before their titer reached 0.1 c.c. Animals No. 316 and No. 415 were exceptions, showing equivalent or better titers in three and two months, respectively. As difficult as it was to get data on female guinea pigs, what has been indicated about the males, in general, held true for females.

Litter Mates.—In considering *female litter mates* raised in different cages, it is difficult to obtain significant results, largely because of the interference of pregnancy with complement activity. Additional complications arose where litters contained both males and females. However, some information may be obtained by a comparison of litters 3 and 4 in Table II. All of these animals were females except one male in litter 4 that died after the first bleeding. Two of these in litter 4 had a higher initial titer than those of litter 3 and tended to maintain this throughout the experiment. One in each litter was raised in cage 1 inside where identical conditions of maintenance did not seem to equalize their titers. Complement titers of all animals were low at the beginning, but variations among the same litters were noticeable. Litter 5, No. 403, had a higher titer than No. 190 for nine bleedings, twice was of equal titer and twice lower.

*Exception occurred in cage 1 inside where all male animals died at least four months before the experiment was completed, and in cage 3 outside where one female did give a decidedly higher titer at the last bleeding.

†Litters 10, 11, and 12 were added August 31, 1933, to replace animals that died.

In *comparing female and male litter mates,* the males invariably reached and maintained a higher titer, although this comparison may be unfair because of pregnancy. One female of litter 6 (No. 191) lived at least the last four months without being pregnant, and her titer never equalled that of the two males of the same litter.

Only in litter 6 did two *male litter mates* live to the completion of these studies, and after six months their complement strengths were nearly parallel. In litter 7, where seven comparable titers of two males were obtained, the titers again paralleled one another. All members of both litters (6 and 7) were raised under different conditions (Table II). Definite conclusions regarding male litter mate comparisons can hardly be reached because the number of comparisons were too few. All animals came from good stock and all males had reached a titer of 0.1 c.c. by the time they had been on test eight months or less.

Feed.—In preparing the three diets, no attempt was made to feed deficient food, but rather to use rations successfully used in laboratories where pigs are raised as a source of complement. Data on females on the various rations are again uncertain for reasons already discussed. As indicated in Table I, a titer of 0.12 c.c. was obtained only twice, both in the same pig, on ration 1, housed outdoors, and in only one instance on the same ration indoors. On ration 2 outside, the two females had two titers each of 0.12 c.c., and once one had a titer of 0.10 c.c. at a time when she had just previously given birth to young. The one female on ration 2 inside twice had a titer of 0.12 c.c. One female on ration 3 outside had a titer of 0.12 c.c. four times, and a cage mate, only once. On ration 3 inside a titer of 0.12 c.c. was reached only once.

On ration 3, one male of litter 6 indoors and one of this litter outdoors, and another male outdoors reached a titer of 0.10 c.c. by the second, fifth, and fourth month respectively. Males on ration 2 took longer to reach such titers, two indoors requiring eight months, and the two outside, six and seven months. The three males on ration 1 inside all died before the experiment was completed, only one having a titer of 0.10 c.c., reaching this in four months. The three males on ration 1 outside acquired a titer of 0.10 c.c. by the sixth month.

Apparently males fed ration 3 showed some slight superiority over those fed other rations. We would favor this ration because it is well balanced, easily handled, reasonably cheap, and necessitated green feed only every other day, and because all males on this diet showed a high titer in a reasonable time.

Sunlight and Temperature.—These factors apparently did not have any appreciable effect on complement titer of male and female guinea pigs. However, males in cage 2 inside required a little longer to reach a satisfactory titer than any others, while the male in cage 3 inside was the first to reach a titer of 0.1 c.c. The males in cage 3 outside reached a satisfactory titer a little in advance of the other outside cages.

Animals housed outdoors were kept there regardless of the weather. The young animals did very well through the hot summer months when temperatures of 100° F. were not uncommon. However, they preferred the shade and thus cannot be compared to the results of Remlinger and Bailly[20] who found rodents may die if left in the sun for as short a period as half an hour. During December, 1935, and January and February, 1936, the temperature was

usually near freezing, and subzero weather with unusually heavy snowfall was not uncommon. For this entire period the guinea pigs were kept outdoors except from January 23 to February 26, when they were housed in an unheated barn. This extreme weather had no effect upon complement as the titers (Table I) were always equal to and sometimes better than in the corresponding ages inside kept at a rather uniform temperature throughout the year.

After this work was completed, the temperature reached 104° F. with high humidity prevalent, between July 6 and July 10, 1936, and six of the animals died in the outdoor group that had been allowed to stay outside and treated as though on experiment, while no deaths occurred indoors. Postmortem examinations revealed only congested lungs, probably due to rapid respiration, indicating that the pigs had died of suffocation, probably as a result of intense heat and high humidity. Had the pens been larger with more space for the circulation of air, this might not have happened. A year before the young animals were exposed to heat as intense as that encountered in 1936, but were apparently able to withstand the excessive heat better than the mature animals.

It is noteworthy that wide differences in temperature had no effect upon complement strength, although it is not recommended as a result of this work that animals be reared in outdoor pens. The animals did survive extreme cold very well, and with larger and better aerated pens, sheltered from the sun, could probably have survived the excessive heat, although there are insufficient data upon this point.

DISCUSSION

The effect of sex of guinea pigs upon complement titer could not be accurately determined from these experiments, largely due to pregnancy radically lowering titers. This effect of pregnancy differs from Rich,[1] who reported pregnancy did not affect titer, and from the results of Kolmer,[8] confirms the results of Griffith and Scott quoted by Browning,[4] and justifies the recommendations of Kolmer,[8] Cummer,[9] Simmons,[11] and others.[13, 14]

As the guinea pigs became older the complement titer of males greatly increased, six of eleven living until completion of the studies showed no drop in titer during the last several months, and the drops manifested by five were probably individual variations, since their distribution could not be correlated with other factors. This increased titer of mature males is contrary to the report of Hyde,[2] but agrees with the work of Osborn[6] on rats. Our results showing low titers of young animals, also confirmed by repeated examination of other week-old guinea pigs, might be considered to agree with Friedberger and Gurwitz[5] who reported that newborn animals may have the same titer as the mother, since, at the time of birth of the young, the mothers usually had a very low titer. Findlay, Fua, and Noeggerath, according to Browning,[4] found a marked increase during the first three months of life, which our results substantiate, although we found most males must be five to seven months old before reaching a titer of 0.1 c.c., even though some animals reached that titer at three or four months.

Guinea pigs should weigh not less than 450 gm., and preferably 600 gm. or more before the most potent titer is reached according to our results. Cummer's[9] recommendations that guinea pigs weigh 400 gm. or more before use for complement compare closely with our results. The use of larger animals is indi-

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cated however and Simmons[11] definitely stated that full-grown healthy males should be used. The authors' results are somewhat contrary to Friedberger and Gurwitz[7] who found that differences in complement titer of animals between 150 and 600 gm. were independent of age and weight. Kolmer[8] found little difference in hemolytic activity between a group of 200- to 280-gm. animals and another averaging 400 to 580 gm. when fed, bled, and the serums treated alike; but since the sex and titer were not given, our results could not be reconciled.

The recommendation of the United States Department of Agriculture[18] and Austin[19] were followed in that diets were well balanced. Rich,[1] Reudiger,[3] and Browning[4] were of the opinion that diet had little effect upon complement content. Kolmer[8] stated that a mixed diet, maintaining animals in good health, was sufficient to yield acceptable complement, and in general, we reached the same conclusion after feeding animals for a year. Simola and Brunius[15] reported that neither A or C avitaminosis nor the feeding of vitamin D would affect the potency of guinea pig complement. The present work cannot be compared with that on vitamins, but the results would tend to show the probable lack of effect of feed on complement.

Guinea pigs are generally raised indoors. A report of the United States Department of Agriculture[18] points out that the temperature should not drop below 65° F. For no apparent reason, the majority of the animals which did not survive the experiment were housed indoors. The outdoor group all survived exposure for some time to subzero weather, and when young, survived extreme heat in summer. As far as complement titer was concerned little difference was noted between titers of those raised in outdoor pens and indoors.

CONCLUSIONS

Young guinea pigs consistently had low, unsatisfactory titers.

All male guinea pigs gradually increased in complement titer between the third and sixth month, while females almost invariably had a lower titer than males after the fourth to sixth month due to pregnancy. This interference of pregnancy, usually not considered by others, made it difficult to reach conclusions on litter mates, age, weight, feeding, sunlight, and temperature.

Male animals did not usually reach a titer of 0.1 c.c. until five to seven months old, and this titer was not reached by 57 per cent of the males examined until they weighed 600 gm. or more.

Comparisons of litter mates were also obscured by pregnancy, variation occurring in females of the same litter as well as between litters. In limited observations on males, the titers within the same litter agreed rather closely, with the usual differences between litters. With such variations between individual females of the same litter and different litters and so few comparisons of males, any significance of litter mates could not be evaluated.

Complement titers of guinea pigs maintained for more than a year upon one of three commonly used rations indicated the probable lack of effect of a satisfactory diet upon complement, although males reached a good titer earlier upon the complete commercial feed supplemented by cabbage every other day.

The complement titer of guinea pigs reared and kept outdoors was not affected by extreme cold during winter. Likewise the titer was not influenced in summer, although many mature animals died from suffocation during excessive heat and humidity.

Our results suggest that the most satisfactory complement should be obtained from male guinea pigs, usually not less than five to seven months old, ordinarily weighing 600 gm. or more, and that pregnant animals are undesirable sources of complement.

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LABORATORY METHODS

DIFFRACTION METHODS FOR MEASURING THE DIAMETER OF THE RED BLOOD CELL*

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THE measurement of the size of the erythrocyte is a valuable procedure in a study of the blood dyserasias.[1-3] The determination of the mean erythrocyte volume is a simple test and has become a part of the routine blood study in many laboratories. The mean erythrocyte volume affords the most satisfactory basis for a laboratory classification of the anemias, and often gives information concerning etiology and the effect of treatment.

The determination of the diameter of the erythrocyte as a measure of the size of the erythrocyte has been advocated by many workers. Price-Jones,[4] in numerous publications, has demonstrated the clinical value of the distribution curve of the erythrocyte. By measuring a large number of cells (200 to 1,000), the range of diameters, which indicates the degree of anisocytosis as well as the mean diameter, is obtained. Price-Jones projects the cells with a camera lucida at a known high magnification and measures the image directly with a millimeter rule. Most workers have used an ordinary micrometer eyepiece. I have employed a filar micrometer as the movable hair facilitates measurement. Another very satisfactory method is the direct projection of the blood film on the ground glass plate of a photomicrographic camera at a fixed high magnification and direct measurement of the image with a millimeter rule. The measurement of a sufficiently large number of cells to give an accurate determination of the mean diameter and to construct a distribution curve is a laborious and time-consuming procedure. This has prevented any extensive use of direct measurement for determining cell size. The volume of the cell is also a much more sensitive indicator of cell size than is the diameter. This is well illustrated by the findings in pernicious anemia. Here the average increase in diameter of 14 per cent corresponded to a 46 per cent increase in volume.

The cell diameter is an accurate indicator of cell volume provided any variation in cell morphology affects diameter and thickness equally. In pernicious anemia and in some other clinical states, this is usually true. In the microcytic hypochromic anemias, however, there may be no reduction or even an increase in diameter with a marked decrease in volume. This can be explained only on the basis of a great decrease in thickness. In congenital hemolytic icterus, there is characteristically a dissociation of the normal relation of diameter and volume since the cells tend to be spherical and so are much thicker than normal. In such conditions, there is a direct relation of the tend-

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ency to spherocytosis to the characteristic increased fragility in hypotonic salt solutions.[5]

Knowledge of the mean cell diameter, however, is a valuable addition to the blood study in many cases and in certain conditions is essential. If both the volume and diameter are known, the thickness can be calculated and a cross-section of the mean cell visualized. In order to make the determination of the cell diameter a simple, rather than a laborious procedure, the diffraction method has been utilized. The availability of the diffraction method of measuring erythrocytes depends on the fact that a blood film placed in a beam of polychromatic light breaks it up into successive bands of its component colors. The red cells act as opaque disks on a screen just as do the apertures of a diffraction grating. The size of the halo depends on the diameter of the red cells and its distance from the light. It varies inversely as the diameter of the erythrocytes and also inversely as its distance from the light. Thomas Young, in 1813, first showed that the principle of diffraction could be used to measure small objects. He constructed an instrument which he called an eriometer or wool-measurer and with which he measured red cells, wool hairs, and vegetable spores. No practical application of Young's discovery was made until Pijper independently rediscovered[7] the application of the principle of diffraction to the measurement of small objects. It is due largely to the work of Pijper that diffraction methods have been made practical and popular. Recently Pijper's instrument has been manufactured by Zeiss as "Pijper's blood-cell-tester."[8] This apparatus has a built-in source of light which requires no adjustment. There is a horizontal stage on which two blood smears can be placed. The diffraction patterns appear on a ground-glass screen which forms the top of the apparatus. A partition is placed in the middle of each diffraction pattern so the remaining semicircles appear in close apposition on the screen, thus facilitating comparison. Each diffraction pattern consists of a bright center surrounded by an orange ring and then follow the colors of the spectrum in natural order, starting with red. After that green and red circles alternate with one another. The mean diameter is determined by measuring the diameter of the yellow ring. The greater the number of macrocytes, the smaller and more intense the violet ring. An increase in microcytes results in a larger red ring. The diffraction pattern thus shows not only the mean diameter but also the anisocytosis and the frequency of macrocytes and microcytes. Direct measurement of the violet ring is made from the inner edge and of the red ring from the outer edge. The distance between the violet and red rings measures the degree of anisocytosis.

Emmons, in 1927,[9] described a simple instrument based on Young's method for measuring the mean diameter of erythrocytes. It consists essentially of two telescopic tubes. The larger, 10 inches in length, is fitted with two small batteries and with electric bulb and switch like an ordinary flashlight. Immediately in front of the electric filament is placed a disk perforated near its periphery by a ring of holes and with one hole at its center. By this arrangement, direct illumination is obtained through the central hole and an indirect and consequently dimmer light from the holes at the periphery. The other tube, which telescopes completely into the first, carries the blood slide and is

graduated to read off directly the diameter of the red cells on the film. Emmons designated this instrument the eriometer, using the name given by Thomas Young to his instrument employing the same physical principles. Unfortunately, this instrument has never been made available commercially. To operate the instrument, a blood film is placed in the slot provided for it. The telescopic tube is adjusted to make the red halo overlie the peripheral ring of holes. The diameter of the cells is then read off directly from the scales on the inner tube.

Eve, in 1929,[10] made an instrument for measuring the diameter of red cells which he called the "halometer." This instrument also is based on the diffraction principle.

Bock, in 1933,[11] described an "erythrocytometer" for measuring red cells, again employing the principle of diffraction. In this instrument, the beam of light passes through two apertures so a blood film placed in the beam gives two spectra. A sleeve is adjusted until the outer red bands of the two spectra touch tangentially, and the cell diameter is read off directly from a scale on the supporting tube. Pijper[12] criticizes Bock for using the red spectral ring for measurement since, he says, this is an expression of the smallest cells in the blood film as is easily demonstrated with his apparatus. The red halo is satisfactory for measuring the mean diameter of normal films since the anisocytosis is relatively slight, but in the anemias the anisocytosis might be marked, so the size of the red circle should not be used to measure the mean diameter. This criticism of Pijper probably explains the unsatisfactory readings we have often obtained with the Bock erythrocytometer in pernicious anemia and the extreme microcytosis of congenital hemolytic icterus.

Diffraction methods for measuring erythrocytes have also been described by Millar[13] and Falisi.[14] None of the diffraction methods necessarily give the true mathematical average of cell diameters. The intensity of a halo at any given point is directly proportional to the number of cells of the corresponding diameter, so the point of maximum intensity indicates the mode or characteristic diameter of all the cells present. The mode gives as much information, however, as does the true mean diameter.

In 1931,[15] I reported a comparison of measurements made with the Emmons eriometer and with a filar micrometer. I also calculated the diameter in each instance from the mean cell volume using Warburg's nomogram,[16] which is correct only when the relation between diameter and thickness remains constant. I concluded that the value of determining the mean diameter did not justify the time required for the procedure when direct measurement is employed. The readings obtained with the Emmons instrument checked closely with those obtained by direct measurement. The eriometer readings were made on the same stained film used for the filar micrometer measurements.

It has become increasingly evident that measurement of the mean diameter of the red cell is of value if not too time-consuming. Thus the fragility of the red cell in congenital hemolytic jaundice has been shown to be dependent on the shape of the cell, which can be determined only when the diameter as well as the volume is known.[5] I have compared readings made on the same film by Emmons', Pijper's and Bock's methods with direct measurement and with calculation of the diameter from the volume readings. The results show that very ac-

curate readings are obtained with the Emmons eriometer and the Pijper blood tester. The readings with the Bock erythrocytometer are not constantly accurate in the abnormal cases. The Emmons instrument is unfortunately not available on the market. We now use this instrument routinely in all blood studies since no dark room is required; it is portable, and gives accurate readings.

Pijper[8] suggests that his instrument be used qualitatively as well as quantitatively. Since an abnormal film can be compared directly with a normal blood film, one may detect a variation from normal immediately. He also emphasizes the importance of the red and violet rings in detecting anisocytosis and the relative number of large cells and small cells. He differentiates three types of patterns:

Type I: Violet, red, and yellow rings, all small. Distance between violet and red rings relatively large. This pattern means macrocytosis and pronounced microcytosis typical of pernicious anemia.

Type II: Yellow ring of normal diameter which means normal mean diameter of cells. Violet ring is smaller, and red ring is larger than normal, indicating more anisocytosis than normal. The distance between the violet and red rings measures the degree of anisocytosis.

Type III: All rings larger than normal and distance between violet and red rings often relatively increased. This is the typical pattern of congenital hemolytic icterus (spherocytic anemia) and the microcytic anemias due to iron deficiency.

TABLE I

COMPARISON OF DIFFRACTOMETRIC WITH DIRECT MEASUREMENTS OF STAINED FILMS OF NORMAL BLOOD

NO.	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY					CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX
		DIRECT MEASUREMENT	EMMONS ERIOMETER	PIJPER BLOOD-CELL-TESTER	BOCK ERYTHROCYTOMETER	CALCULATION FROM MEAN CELL VOLUME		
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	
1	92	7.6	7.6	7.1	7.6	7.7	2.05	1.06
2	89	7.3	7.4	6.9	7.3	7.6	2.15	1.15
3	91	7.5	7.7	7.1	7.5	7.7	2.10	1.06
4	90	7.8	7.6	7.1	7.8	7.7	1.90	0.96
5	90	8.0	8.1	7.4	8.0	7.7	1.85	0.90
6	92	7.6	7.6	7.2	7.6	7.8	2.15	1.06
7	89	7.4	7.5	7.0	7.4	7.7	2.10	1.12
8	88	7.7	7.6	7.1	7.7	7.6	1.90	0.95
9	87	7.6	7.7	7.2	7.6	7.5	1.90	0.99
10	96	8.0	7.7	7.2	8.0	7.8	1.90	0.95
Average	90	7.6	7.6	7.1	7.6	7.7	2.00	1.02

In making the comparative measurements, I have measured only the yellow ring to determine mean cell diameter in the Pijper blood-cell-tester. The diameter of the cells in a blood film may vary greatly, depending on the thickness of the spread. The measurements of stained films have all been made on cover glass preparations in which the cells just touch but do not overlap. Films made on slides were used for the measurement of unstained preparations. Areas were selected where the cells were properly spread and did not overlap. The halos are much brighter on unstained films and so can be read more easily. In a stained

TABLE II

COMPARISON OF READINGS OF STAINED AND UNSTAINED FILMS OF NORMAL BLOOD

NO.	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY							CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX
		EMMONS ERIOMETER		PIJPER BLOOD-CELL-TESTER		BOCK ERYTHRO-CYTOMETER		CALCULATION FROM MEAN CELL VOLUME		
		STAINED	UNSTAINED	STAINED	UNSTAINED	STAINED	UNSTAINED			
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	
1	88	7.6	7.5	7.1	7.7	7.7	7.7	7.6	2.00	1.00
2	91	7.8	7.6	7.3	7.9	7.7	7.6	7.7	2.00	0.97
3	91	7.7	7.8	7.1	7.8	7.7	7.7	7.7	1.90	1.01
4	85	7.3	7.3	7.2	7.5	7.6	7.3	7.6	2.00	1.11
5	92	7.8	7.7	7.2	7.7	7.7	7.7	7.7	2.00	0.98
6	84	7.6	7.6	7.2	7.8	7.8	7.7	7.5	1.85	0.96
7	88	7.8	7.8	7.2	7.9	7.7	7.8	7.6	1.85	0.96
8	82	7.6	7.5	6.9	7.6	7.7	7.7	7.5	1.90	0.95
9	88	7.7	8.0	7.2	8.0	7.8	7.9	7.7	1.75	0.98
10	91	7.8	7.8	7.1	7.8	7.7	7.8	7.7	1.90	0.99
Average	88	7.7	7.7	7.2	7.8	7.7	7.7	7.6	1.90	0.99

film the stain acts as a filter; hence, the colors of the halo are not just the same as in the unstained preparations. In every case, several measurements have been made and the average taken. Often measurements were made on more than one film. The mean cell thickness and the volume thickness index have been calculated as previously described.[17]

Table I shows the comparative measurements on the stained films only from ten normal persons. All films were made on cover glasses. The diameter as determined by the Pijper instrument is 0.5 mm. less than with direct measurements and with the other instruments. I then compared the different diffraction methods on ten more normals (Table II), using both stained and unstained films. The average results with the different methods on unstained films are almost exactly the same. The results with the eriometer and erythrocytometer also check closely with those found on stained films. The readings with the blood-cell-tester on stained films are again lower than on unstained films. These results show that a correction (about 0.5 mm.) must be made for measurements on stained films when the Pijper instrument is employed.

In Table III are shown the comparative readings on stained films only with a group of patients showing a wide variation in mean diameter. The average diameter with the blood-cell-tester is again lower than with other methods, but when a correction is made, it checks closely with the other methods. The readings with the erythrocytometer are apt to be too low if the mean cell diameter is high, or too high if the mean cell diameter is low. In Table IV are tabulated the readings in a group of patients who had either a low volume index or a small cell diameter. All readings were made on unstained films. Here again the agreement is good except with the erythrocytometer, which does not give correct readings when the diameter varies widely from normal.

In Table V are collected the readings on unstained films when the mean cell volume is high as it characteristically is in pernicious anemia, or the cell diameter is high without a corresponding increase in volume, such as is seen frequently in liver disease. Here again, the readings with the Bock instrument are almost uniformly too low, and the average of 25 readings is below that with the other instruments.

In Table VI are shown the readings in a wide variety of conditions in which the volume index and mean cell diameter are within normal limits. Here there is a close check of the results obtained with the three instruments.

SUMMARY AND CONCLUSIONS

Diffraction methods are satisfactory for measuring the mean diameter of the erythrocyte.

In normal films or where the diameter varies little from normal, readings with Emmons' eriometer, Pijper's blood-cell-tester, and Bock's erythrocytometer check closely with direct measurements.

Readings with the Emmons' eriometer and Pijper's blood-cell-tester give accurate results even when the cells are very large or very small.

Readings with the Bock erythrocytometer tend to be too low if the cells are large and too high if the cells are quite small.

TABLE III

COMPARISON OF DIFFRACTOMETRIC WITH DIRECT MEASUREMENTS ON STAINED FILMS OF ABNORMAL MEAN CELL VOLUME

NO.	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY					CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX	DIAGNOSIS
		DIRECT MEASUREMENT	EMMONS ERIOMETER	PIJPER BLOOD-CELL-TESTER	BOCK ERYTHROCYTOMETER	CALCULATION FROM MEAN CELL VOLUME			
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS		
1	63	7.0	7.1	6.6	7.5	6.8	1.60	0.90	Idiopathic hypochromic anemia
2	59	6.7	7.1	6.7	7.6	6.7	1.50	0.75	Idiopathic hypochromic anemia
3	59	7.0	7.0	6.5	7.3	6.7	1.55	0.79	Idiopathic hypochromic anemia
4	60	6.8	7.1	6.5	7.4	6.7	1.50	0.77	Idiopathic hypochromic anemia
5	68	7.2	7.3	6.9	7.4	7.0	1.60	0.88	Idiopathic hypochromic anemia
6	59	7.2	7.5	6.9	7.6	6.7	1.35	0.72	Lead poisoning
7	84	7.8	7.6	7.2	7.9	7.5	1.80	0.98	Idiopathic hypochromic anemia
8	84	7.7	7.5	7.0	7.5	7.5	1.90	0.90	Idiopathic hypochromic anemia
9	80	7.4	7.5	6.7	7.4	7.4	1.80	0.87	Idiopathic hypochromic anemia
10	90	7.7	7.6	7.1	7.5	7.7	2.00	0.95	Idiopathic hypochromic anemia
11	89	7.8	7.6	7.2	7.6	7.7	2.00	0.94	Lead poisoning after treatment
12	144	8.9	8.8	8.4	8.4	9.0	2.40	1.07	Pernicious anemia
13	130	8.8	9.2	8.8	8.4	8.7	2.00	0.80	Pernicious anemia
14	92	8.6	8.6	8.6	8.6	7.7	1.60	0.74	Obstructive jaundice
15	93	8.2	8.4	8.1	8.5	7.7	1.70	0.80	Obstructive jaundice
16	105	7.9	8.3	7.9	8.2	8.1	1.90	0.82	Hemolytic anemia
17	97	7.7	7.7	7.2	7.6	7.9	2.10	1.08	Spherocytic jaundice
18	86	7.2	7.3	6.9	7.4	7.6	2.10	1.13	Spherocytic jaundice
19	90	6.2	6.3	5.9	6.6	7.7	2.90	1.82	Spherocytic jaundice
20	160	6.4	6.5	5.9	6.6	9.3	4.80	2.67	Spherocytic jaundice
21	77	6.4	6.4	5.9	6.6	7.3	2.40	1.35	Spherocytic jaundice
22	78	6.5	6.6	6.2	6.9	7.4	2.30	1.24	Spherocytic jaundice
23	110	6.5	6.4	6.2	6.8	8.2	3.40	1.93	Spherocytic jaundice
24	87	6.6	6.4	6.1	6.6	7.7	2.70	1.53	Spherocytic jaundice
25	87	6.9	6.6	6.4	6.7	7.7	2.55	1.38	Spherocytic jaundice
Average		7.4	7.4	7.0	7.5				

TABLE IV

COMPARISON OF DIFFRACTOMETRIC MEASUREMENTS WITH DIFFERENT INSTRUMENTS ON UNSTAINED FILMS OF BLOOD WITH DECREASED MEAN CELL VOLUME

NO.	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY						DIAGNOSIS
		EMMONS ERIOMETER	PIJPER BLOOD-CELL-TESTER	BOCK ERYTHRO-CYTOMETER	CALCULATION FROM MEAN CELL VOLUME	CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX	
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS		
1	85	7.1	7.1	7.6	7.5	2.15	1.16	Chronic pharyngitis
2	77	6.7	6.7	6.9	7.3	2.20	1.31	Spherocytic anemia
3	78	7.8	7.7	7.8	7.3	1.65	0.83	Idiopathic hypochromic anemia
4	86	7.2	7.3	7.6	7.5	2.15	1.13	Hypothyroidism
5	85	7.1	7.1	7.4	7.5	2.20	1.16	Essential thrombopenia
6	61	7.7	7.6	7.8	6.3	1.30	0.68	Banti's disease
7	77	7.4	7.3	7.4	7.3	1.80	0.96	Sarcoma of femur
8	77	7.7	7.7	7.9	7.3	1.65	0.85	Idiopathic hypochromic anemia
9	82	7.5	7.5	7.6	7.4	1.85	0.97	Nutritional deficiency disease
10	61	7.8	7.7	7.8	6.3	1.30	0.64	Idiopathic hypochromic anemia
11	62	7.7	7.6	7.8	6.3	1.35	0.69	Banti's disease with hemorrhage
12	72	7.1	7.2	7.3	7.1	1.80	1.00	Pemphigus
13	58	8.1	8.0	8.0	6.7	1.15	0.56	Idiopathic hypochromic anemia
14	75	7.7	7.8	7.6	7.3	1.85	0.83	Hemorrhage from duodenal ulcer
15	68	7.8	7.6	7.7	7.0	1.65	0.73	Idiopathic hypochromic anemia
16	56	7.5	7.1	7.8	6.6	1.30	0.69	Idiopathic hypochromic anemia
17	75	6.4	6.7	7.1	7.3	2.25	1.43	Spherocytic anemia
18	75	7.7	7.6	7.6	7.3	2.00	0.83	Hemorrhage from duodenal ulcer
19	65	7.3	7.3	7.4	6.9	1.60	0.85	Idiopathic hypochromic anemia
20	89	7.3	7.3	7.3	7.6	2.15	1.14	Chronic tonsillitis
21	87	7.3	7.3	7.3	7.5	2.10	1.12	Idiopathic hypochromic anemia
22	69	7.6	7.5	7.6	7.0	1.85	0.80	Idiopathic hypochromic anemia
23	65	8.0	8.0	7.8	6.9	1.30	0.65	Peptic ulcer
24	63	8.1	8.2	8.0	6.8	1.20	0.58	Idiopathic hypochromic anemia
25	64	7.8	7.7	7.8	6.9	1.35	0.70	Chronic hemorrhage
Average		7.5	7.5	7.6				

TABLE V

COMPARISON OF DIFFRACTOMETRIC MEASUREMENTS WITH DIFFERENT INSTRUMENTS ON UNSTAINED FILMS OF BLOODS WITH INCREASED MEAN CELL VOLUME OR INCREASED MEAN CELL DIAMETER

NO.	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY				CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX	DIAGNOSIS
		EMMONS ERIOMETER	PIJPER BLOOD-CELL-TESTER	BOCK ERYTHRO-CYTOMETER	CALCULATION FROM MEAN CELL VOLUME			
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS		
1	103	7.7	7.8	6.6	8.0	2.25	1.12	Hemolytic jaundice
2	128	8.8	9.0	8.4	8.7	2.10	0.92	Pernicious anemia
3	95	8.2	8.3	7.9	7.8	1.80	0.84	Nutritional deficiency disease
4	96	7.4	7.6	7.4	7.8	2.25	1.21	Pernicious anemia under treatment
5	96	8.7	8.8	8.4	7.8	1.60	0.74	Cirrhosis of liver
6	124	9.5	9.4	8.9	8.6	1.75	0.73	Pernicious anemia
7	122	8.6	8.6	8.1	8.6	2.10	1.00	Hemolytic anemia
8	103	8.4	8.4	8.1	8.0	1.85	0.88	Pernicious anemia ?
9	83	8.3	8.4	8.1	7.5	1.55	0.74	Carcinoma of liver
10	99	8.0	8.0	7.9	8.0	1.95	0.98	Herpes zoster
11	108	7.9	8.1	8.0	8.2	2.20	1.11	Pernicious anemia under treatment
12	81	8.9	8.8	8.4	7.4	1.30	0.58	Cirrhosis of liver
13	96	8.6	8.7	8.4	7.9	1.65	0.78	Cirrhosis of liver
14	84	8.0	8.0	7.8	7.5	1.70	0.84	Pernicious anemia under treatment
15	104	8.4	8.5	8.3	8.1	1.85	0.91	Pernicious anemia under treatment
16	92	8.9	9.0	8.6	7.8	1.50	0.67	Cirrhosis of liver
17	94	8.3	8.4	8.1	7.8	1.75	0.80	Cirrhosis of liver
18	97	7.7	7.7	7.9	7.9	2.10	1.08	Pernicious anemia under treatment
19	100	7.9	7.9	8.0	8.0	2.05	1.03	Pernicious anemia under treatment
20	96	8.1	8.2	8.0	7.9	1.85	0.96	Pernicious anemia ?
21	113	9.1	9.2	8.7	8.3	1.75	0.76	Pernicious anemia
22	100	8.3	8.4	8.4	8.0	1.85	0.89	Pernicious anemia
23	120	8.9	9.0	8.6	8.5	1.90	0.90	Pernicious anemia
24	119	9.0	9.2	8.8	8.5	1.85	0.83	Hemolytic jaundice
25	96	7.9	8.1	7.9	7.9	1.95	1.00	Pernicious anemia
Average		8.4	8.5	8.2				

TABLE VI

COMPARISON OF DIFFRACTOMETRIC MEASUREMENTS WITH DIFFERENT INSTRUMENTS ON UNSTAINED BLOOD FILMS OF ANEMIAS WITH NORMAL MEAN CELL VOLUME

NO.	MEAN CELL VOLUME BY HEMATOCRIT	MEAN CELL DIAMETER BY				CALCULATED MEAN CELL THICKNESS	VOLUME THICKNESS INDEX	DIAGNOSIS
		EMMONS ERIOMETER	PIJPER BLOOD-CELL-TESTER	BOCK ERYTHRO-CYTOMETER	CALCULATION FROM MEAN CELL VOLUME			
	CUBIC MICRONS	MICRONS	MICRONS	MICRONS	MICRONS	MICRONS		
1	90	7.6	7.5	7.6	7.7	2.00	1.04	Pernicious anemia in remission
2	90	7.5	7.6	7.5	7.7	2.05	1.08	Osteomyelitis
3	86	7.6	7.7	7.7	7.6	1.90	1.00	Chronic bronchitis
4	84	7.7	7.7	7.7	7.5	1.80	0.93	Progressive central muscular atrophy
5	85	7.7	7.7	7.7	7.6	1.80	0.95	Dyspituitarism
6	91	7.5	7.7	7.7	7.7	2.05	1.09	Bronchial asthma
7	88	7.7	7.7	7.7	7.7	1.90	0.97	Cystitis
8	84	7.8	7.7	7.9	7.5	1.80	0.90	Pernicious anemia in remission
9	84	7.7	7.9	7.8	7.5	1.80	0.93	Pernicious anemia in remission
10	91	7.7	7.7	7.8	7.7	1.95	1.01	Nutritional deficiency disease
11	81	7.6	7.7	7.7	7.4	1.80	1.01	Nutritional deficiency disease
12	85	7.7	7.8	7.7	7.6	1.80	0.95	Lead poisoning
13	91	7.3	7.6	7.5	7.7	2.20	1.22	Obstructive jaundice
14	83	7.4	7.3	7.5	7.5	1.95	1.04	Peripheral neuritis
15	81	7.4	7.5	7.5	7.4	1.90	1.02	Myxedema
16	85	7.7	7.7	7.8	7.6	1.80	0.95	Spastic colon
17	85	7.4	7.5	7.5	7.6	2.00	1.06	Gallstones
18	85	7.4	7.6	7.4	7.6	2.00	1.07	Pernicious anemia in remission
19	84	7.5	7.7	7.5	7.5	1.90	0.98	Pernicious anemia in remission
20	87	7.9	7.9	7.8	7.6	1.75	0.90	Pernicious anemia in remission
21	84	7.8	7.5	7.7	7.5	1.75	0.90	Nutritional deficiency disease
22	95	7.7	7.7	7.7	7.8	2.05	1.05	Pernicious anemia in remission
23	86	7.4	7.5	7.5	7.6	2.00	0.92	Diabetes mellitus
24	84	7.8	7.7	7.7	7.5	1.75	0.94	Hypertension
25	88	7.7	7.8	7.6	7.6	1.90	0.97	Hypometabolism
Average		7.6	7.6	7.6				

The Emmons' eriometer has the advantage of portability and not requiring a dark room.

The Pijper blood-cell-tester is excellent for comparing an abnormal with a normal film and also indicates the degree of anisocytosis as well as the mean diameter.

The readings are the same with stained as unstained films with the Emmons and Bock instruments.

With the Pijper instrument, the diameter measures about 0.5 mm. less on a stained than on an unstained film.

For direct measurements, a film projected on the ground glass of a photomicrographic camera is very convenient. The filar micrometer is the most satisfactory ocular micrometer.

The calculation of the diameter from the mean volume by Warburg's nomogram is correct only when the variation from normal affects diameter and thickness equally.

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ERYTHROCYTE SEDIMENTATION AND ANEMIA

A Preliminary Report

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IT IS the purpose of this communication to present and attempt to substantiate by clinical evidence the claim that the relative number of circulating erythrocytes in the blood plasma has very little, if any, influence per se on the *speed of settling* of the red cells. It is further claimed that any effect that may exist is so varying in degree within the same individual, and differs so greatly among different individuals, that any attempt at "correction" of the sedimentation rate in a secondarily anemic patient in large part obscures the true nature of the curve of settling, and thus reduces the value of the reading by introducing an extraneous and, above all, an essentially inconstant factor.

The term "secondary anemia" is stressed, as reference is made only to a diminution in the relative number of circulating red cells as caused, for example, by hemorrhages or by peripheral destruction of normally formed cells as in malaria. Diseases of the blood-forming organs, such as the leucemias and pernicious anemia, are considered in this connection not as forms of anemia proper, but as disease entities having a marked effect on the sedimentation rate independent of the diminution in the number of circulating red cells. For the purpose of substantiating the claims herein put forth, the most clear-cut example would be a loss of blood without hemorrhage into peroral body cavities or tissues, such as might occur in a penetrating gastric ulcer, for the following reasons: a gastric ulcer does not, as a rule, have a noticeable effect on the sedimentation rate in itself, so that hemorrhage from this source represents a loss of blood pure and simple, such as may be encountered in actual clinical experience. Reference is made to interstitial hemorrhage and other extracirculatory accumulations of blood within the body (exclusive of the digestive tract), as these factors are likely to affect the sedimentation rate as a result of the tissue reaction and absorption of abbau products.

An example of the dovetailing of various factors, on the other hand, is to be found in cases of anemia accompanying therapeutic malaria in general paresis, in which the syphilitic agent, the malarial agent, the hyperthermia per se, and possible complicating factors such as decubitus ulcers, etc., all play a part in altering the sedimentation rate, regardless of whether there is an accompanying anemia or not (which, in turn, may be the direct result of either the paresis, or the malaria, or both).

The above examples represent opposite extremes, indicative of the type of material and the pitfalls encountered by the worker in this field. The sedimentation readings herein presented in fragmentary form were obtained for the greater part, but not exclusively, from patients inoculated with double tertian

malaria. All cases were, however, well worked up clinically and followed after termination of the malarial treatment, and did not show, as far as observed, any of the few conditions known definitely to *decrease* the rate of settling.[1, 2] Moreover, although roughly representing a specific clinical group, the individual cases have contributed every known type of erythrocyte sedimentation curve, and it was this very observation which first called my attention to the subject matter herein discussed.

In view of the great number of factors influencing the erythrocyte sedimentation rate, as well as the admitted fact that the actual causes for changes in the

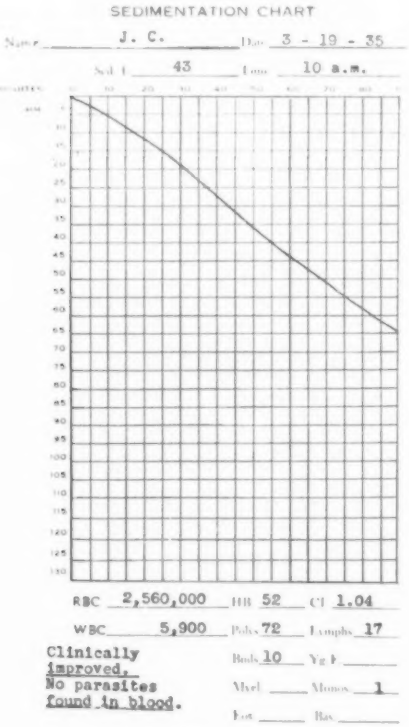
SEDIMENTATION CHART
Name J. C. Date 3 - 19 - 35
Sed. 43 Time 10 a.m.
RBC 2,560,000 HB 52 CI 1.04
WBC 5,900 Poly. 72 Lymphs 17
Clinically improved. No parasites found in blood.
Bands 10 Myel. Monos. 1
Eos. Bas.

Fig. 1.

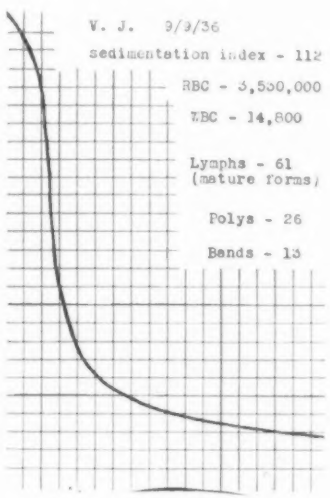

Fig. 2.—V. J., an example of a markedly pathologic curve accompanying a moderately low red cell count in a case of lymphatic leucemia, "aleucemic" form. This patient died a few days after the above reading was obtained.

rate are still at best only in part determined, it would seem that any attempt to eliminate completely and routinely one of the supposed factors, even without a clear knowledge of the exact manner in which the latter might exert an influence, is perhaps premature and certainly represents a tampering with a physiologic phenomenon which in its "unmodified" form is quite capable of diagnostic interpretation in the hands of the experienced observer.[3] The term "unmodified" is purposely put in quotation marks, as I am aware of the fact that, with the usual technique, the test cannot be considered unmodified in the strictest sense of the word. It is, in the first place, a test in vitro, and, second, the blood employed contains an anticoagulant. Furthermore, the average laboratory procedure involves considerable contact with air, and the element of elapsing time between venipuncture and setting up of the blood,[4, 5] although relatively negligible within a more or less definite limit, is rarely given sufficient attention to

render it a constant factor, even when a group of sedimentation rates is read simultaneously. We may, however, disregard at least the factors of the glass container and anticoagulant,[6] as they are present in every instance, and, at least within the limits of the particular technique used (e.g., Linzenmeier, Westergren, Cutler, etc.), remain constant, except for gross errors introduced by faulty manipulation. As a matter of fact, various attempts at correlation have shown that there is no great element of discrepancy involved even in comparing results with the various classical techniques.[7]

At this point it seems appropriate to diverge, in order to review briefly as a group all factors that might have an effect on the blood in vitro, and that have been considered for that reason by various authors. In addition to the form of the glass vessels, the kind of anticoagulant employed, and the time interval between obtaining the blood and setting up the test, factors to which reference has

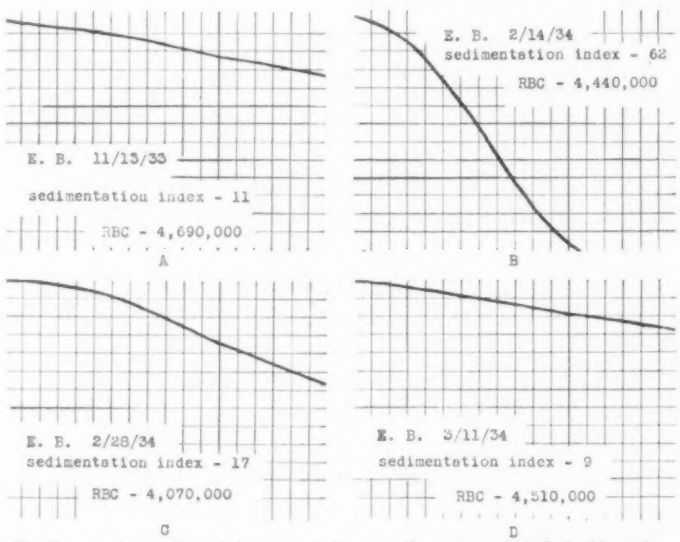

Fig. 3.—E. B., an example of the most frequently encountered finding, i.e., a temporary marked drop of the sedimentation curve with a relatively stable red cell count (acute gangrenous appendicitis, operation, and uneventful recovery in an otherwise healthly nineteen-year-old nurse).

already been made, the following have received attention at the hands of a number of workers: the temperature at which readings are made,[4, 8, 9] the meteorologic conditions,[10] the position of the glass container,[8] etc. It is not within the scope of this report to discuss this aspect of erythrocyte sedimentation in detail, but it may be said at this point that of all the above-named factors, although some of them have been found definitely to affect the rate of settling, none need be considered seriously within the limits of so-called "normal" working conditions beyond the point of avoiding extreme fluctuations in temperature (i.e., maintaining average room temperature) and unnecessary delay in starting the test, using the same anticoagulant consistently, setting up the tubes as prescribed for the technique employed, and keeping all glassware properly clean. In other words, it is necessary only to observe the precautions customary in a well-conducted laboratory. This statement would seem to be an invitation to inaccuracy,

but the attitude it represents is based on the most outstanding characteristic of the sedimentation phenomenon, which in turn furnished the basic motive for the presentation of this report.

It seems to the writer that there exists a tendency to lose sight of the fact that the rate of settling of the red blood cells is *essentially a nonspecific biologic phenomenon with only approximate values*, or is so at least as far as our present knowledge goes. It is, for this reason, impossible to draw a sharp line of demarcation between what represents a "normal" and what a "pathologic" reading, as witness the confusingly wide range of so-called "normal limits," as given by different authors.[1, 11-15]

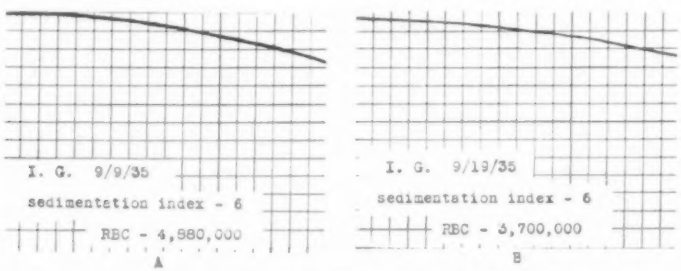

Fig. 4.—I. G., an example of an appreciable drop in the red cell count with an unchanging normal curve, interpreted as "prognosis favorable," in a paretic inoculated with double tertian malaria. Patient's subsequent course satisfactory.

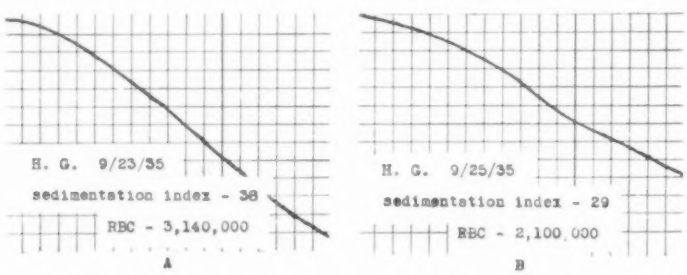

Fig. 5.—H. G., an example of marked and progressive anemia with an only moderately pathologic and improving curve, interpreted as "prognosis favorable," in a paretic with double tertian malaria. Patient's subsequent course satisfactory.

It is in great part this very absence of specificity and of definite normals that has led to the great variety of approaches in an attempt to determine the agents responsible for the fluctuations in the rate of settling. The literature today abounds in references to such widely divergent etiologic factors as: the time of day at which the blood is obtained, the degree of venous stasis present at the time of venipuncture,[16] the effect of meals,[17] endocrine imbalance (e.g., thyrotoxicosis, epinephrine content of the blood, diabetes mellitus,[18] etc.), variations in amount and proportion of the various protein components of the blood plasma (albumin, globulin, fibrinogen, etc.),[16, 19-23] variations in the electric charge of the erythrocytes,[1] variations in lecithin and cholesterol content, as well as of other lipoids, and hyperthermia, irrespective of its cause, to list only a few of the factors of a general character representing pathologic conditions or extreme physiologic fluctuations. In the final analysis, it is possible to correlate with a fair degree of accuracy the degree of increased rate of settling of erythro-

cytes with the amount of cell destruction going on in the body, but this working theory is, after all, only provisional and based on observation rather than complete understanding.

When the problem is approached from the point of view of specific disease entities and their effect on the rate of settling, another grouping is obtained, best represented at present, although necessarily by no means exhaustive in its scope, by the compilation of Cutler.[14] In this compilation, diseases are grouped according to whether they are "accompanied by an abnormal sedimentation rate," whether they "influence the sedimentation rate very little if at all," and as "not influencing the sedimentation rate." There is, unfortunately, no mention of anemias in this list. It may, however, be stated briefly here that pernicious anemia and the various leucemias are admitted to have a very definite and constant effect on the sedimentation rate, which is quite distinct from any effect that might be caused by an uncomplicated secondary anemia (see Fig. 2).

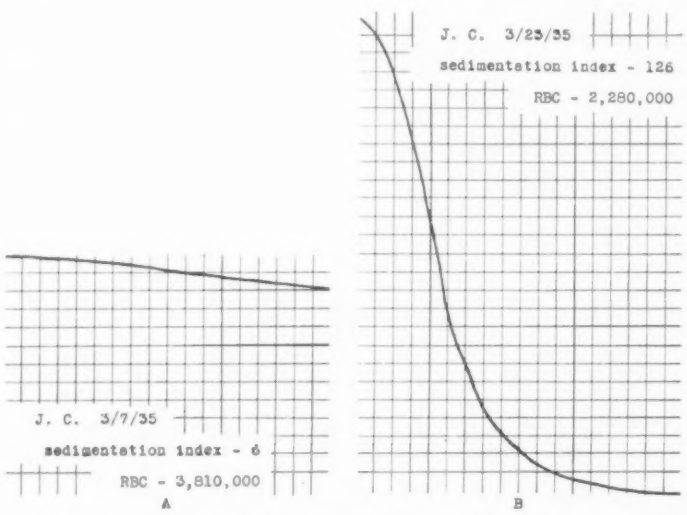

Fig. 6.—I. C., an example of marked and progressive anemia with a sudden change from a normal to an extremely pathologic curve, the latter pronounced even in proportion to the anemia; interpreted as "prognosis grave," in a paretic with double tertian malaria. Patient died within twenty-four hours of obtaining the above reading.

The material herein referred to consists exclusively of sedimentation curves charted graphically, rather than of one-time readings. It is my opinion that, despite indefinite normals, a curve is capable of direct interpretation on the basis of consideration of several factors, such as initial speed of settling, fluctuations in the speed throughout the period of reading, the one-hour "index," the final reading after settling is complete and, last but by no means least, a comparison of repeated tests on the same individual.[4, 24]

Just how much importance may be ascribed to any one or all of these factors is entirely a matter of "habituation" on the part of the physician, that is to say, of the amount of experience acquired by the routine reading of sedimentation curves in conjunction with a consideration of red cell count, white cell count, differential, urinalysis, temperature chart and other laboratory and clinical data. The chart used at Springfield State Hospital, Maryland, during

the years 1934, 1935, and 1936, when the material herein referred to was obtained, was designed by the writer with the above principles in mind (Fig. 1). On this chart were recorded the readings obtained with the Westergren tube, using citrate as an anticoagulant.[6]

In the attempt to augment the utility of the sedimentation phenomenon as a laboratory test, a great many modifications of the original methods have been evolved, but the most conspicuous of these is the socalled "correction" of the rate, intended to compensate for a low red cell count either by subtracting from the reading a number of millimeters in each case supposedly equivalent to a certain volume or number of red cells, or by changing the plasma-cell ratio in vitro before reading.[25-30] These corrections do not, of course, take into account the *type of curve* obtained. In its extreme form, this procedure has led to the adoption of "correction charts." Only the corrected reading thus obtained is referred to for diagnostic interpretation in these modifications. The considera-

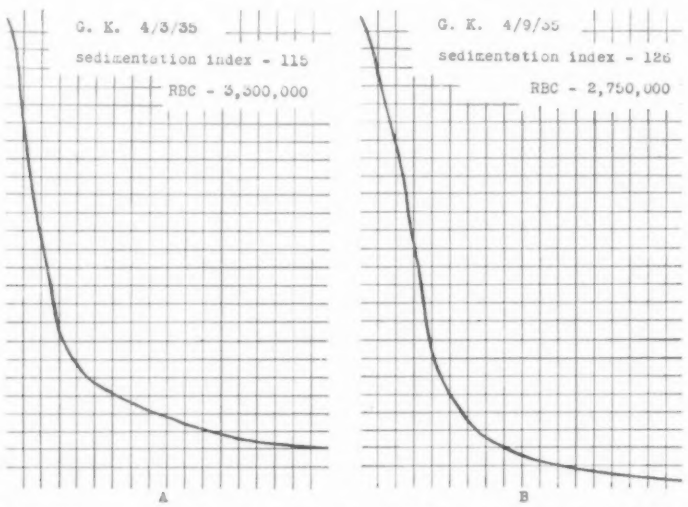

Fig. 7.—G. K., an example of progressive, but less pronounced, anemia with an extremely pathologic curve from the outset, interpreted as "prognosis hopeless," in a paretic with double tertian malaria. Patient died within twenty-four hours of obtaining the above reading.

tion of these methods does not, of course, include correction for the factor of cell volume shrinkage produced by the particular anticoagulant employed.[30]

In the present report, preparatory to demonstration of material, it is maintained that the effect of a moderate (and in some cases a marked) diminution in the number of circulating red cells in the blood has a negligible effect on the sedimentation curve, and that such effect as may be present impairs the diagnostic value of the test far less than the essentially uncertain and even less well understood effect produced by "correcting" the reading. Tests conducted with accurately measured numbers of red cells increased and decreased in vitro,[29] have, to be sure, demonstrated that the sedimentation rate increases apparently in proportion to the number of cells removed from the plasma. Such consistency has *not*, however, been determined to date in blood tested as obtained from the circulation without further manipulation. In other words, attempts at correcting the rate are, at least in the present state of our knowledge, advised

against by the writer as introducing a misleading element of pseudoaccuracy into an essentially inaccurate test. It is contended that the following samples of case material substantiate these claims. The laboratory tests involved were carried out in the course of routine work, with occasional checks and rechecks, and the examples illustrated are purposely chosen as offering sufficiently marked variations from the normal to provide a margin for minor technical errors.

I am aware of the fact that the red cell count in itself is an only approximate indicator of the *volume relationship* between plasma and cells, as well as of the fact that, with a diminished total cell volume, the *final reading* will be quite different from that of normal blood. The statements made in this report have reference exclusively to the *speed of settling*, not to the distance traversed by the red cells before packing occurs. In most of the cases included in this report, twenty-four-hour readings were made, and in some of the earlier cases, packed cell volume determination, but neither item was recorded in every instance, as this material (see above) was not accumulated in the course of experimental work. No figures of this kind are therefore given here. Moreover, attention was paid in every instance to the general appearance of the erythrocytes, both in regard to size and uniformity of shape, in the course of the red cell counts and smear studies, and the graphs chosen for illustration of this report were picked from the standpoint of representing sufficiently wide variations to take into account the factor of fluctuating total cell volume as separate from the cell count.

No attempt will be made within the confines of this report to give complete case findings, or to reproduce the complete series of sedimentation rates for any one individual.

SUMMARY

1. It is contended that the relative number of erythrocytes in the plasma has a negligible effect per se on the rate of settling of the red cells, and that such effect as may exist is inconstant.

2. A number of intrinsic and extrinsic factors that have received attention as possibly affecting the rate of erythrocyte settling is listed, as indicative of the present status of sedimentation as a nonspecific and only partly understood phenomenon.

3. It is maintained that attempts at "correction" of the sedimentation rate by compensating for anemia, and especially the use of "conversion charts," represent pseudoaccuracy and in large part invalidate the readings.

4. It is further maintained that the "unmodified" sedimentation curve, graphically recorded, is a valuable diagnostic aid when correlated with other laboratory and clinical data.

5. A chart is shown for the joint recording of sedimentation curve, sedimentation index, red and white cell count, differential count, and special information.

6. A number of selected curves is reproduced in substantiation of the contention that the sedimentation rate and the red cell count may fluctuate independently of one another, and even move in opposite directions in the same individual, in the course of a pathologic process.

7. If an individual with a low red count, but otherwise essentially normal findings, shows a definitely pathologic sedimentation curve, that individual's true ailment has not been determined and is of a serious nature.

Since writing the above, two articles have come to my attention, one of which appeared in the May, 1937, issue of the J. LAB. & CLIN. MED., the other in the October 16, 1937, issue of the J. A. M. A. The former was entitled "The Sedimentation Rate in Experimental Anemia (Rabbit)," by R. O. Gregg, and the latter "The Erythrocyte Sedimentation Rate," by E. G. Bannick, R. O. Gregg, and C. M. Guernsey, of Rochester, Minn.

The attitude of the authors, as outlined in their joint paper, coincides so perfectly with my own on this subject that I feel mention should be made of their work in this connection. The experiments carried out on rabbits represent, in my opinion, a great step forward in the refutation of the conclusions arrived at by in vitro methods, and in the elimination of too complicated techniques evolved chiefly as a result of such methods and conclusions.

Despite the outlook for eventual general agreement on the factor of anemia in the interpretation of the sedimentation rate, there remain individual differences of opinion on the relative value of graphic curves or one-time readings. This latter difference would appear to be still a matter largely of personal experience, and should not retard the eventually general acceptance of erythrocyte sedimentation readings as a valuable aid to diagnosis and treatment.

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"INTEGRATIVE ACTION" OF MIXTURES OF SIMPLE ORGANIC SUBSTANCES IN OXIDATION-REDUCTION PHENOMENA PRODUCED BY AUTOCLAVING*

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THIS paper considers the persistence of reduction of methylene blue† in mixtures of certain organic acids and sugars at pH 5.6 and 8.0 after autoclaving at 15 pounds pressure for twenty minutes. Such persistence is compared with that of cysteine hydrochloride (Eastman) solutions of varying pH and concentrations, control solutions and d-glutamic acid-cysteine HCl mixtures. "Integrative action" is considered as of three types.

All solutions contained 0.001 per cent methylene blue. The stock solution of methylene blue was made up to 0.1 per cent with distilled water of pH 5.6. Observations and tests were made under aerobic conditions. The solutions were kept at room temperature in diffused light. Solutions were contained in 2 by 17 cm. cotton plugged test tubes. The pH was adjusted colorimetrically before and after autoclaving on solutions to which methylene blue had not been added.

When reduction was persistent it was recorded by the Hardy spectrophotometer‡ at intervals of six to forty-eight hours: the solutions were poured gently into spectrophotometric cells of 1 cm. inner thickness and gently poured back into the test tubes to be reobserved at a later time in a similar manner. The terms complete oxidation and complete reduction as used in this paper signify an oxidation and reduction as complete as recordable by the eye or the spectrophotometer.

The term "integrative action" has been used in describing results since certain mixtures show more persistent reduction than either substance separately.

*From the Department of Biology and Public Health, Massachusetts Institute of Technology.

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†Methylene blue, U. S. P. Medicinal, C. I. 922, total dye content 88 per cent, National Aniline & Chemical Co. Inc., New York.

Therefore, neither substance can be said to catalyze a change in the other or in the methylene blue. Apparently the physical force of autoclaving activates the change.

It frequently has been observed that after autoclaving certain bacteriologic mediums containing methylene blue show temporary reduction to the eye. The factors involved are indeterminable because of complexity of the medium. They probably are of importance since many recent reports indicate that freshly autoclaved medium is best for growth of certain organisms and that reduced potential insures a more secure foothold for better organismal growth.

Ease of oxidation of reducing sugars and transportation of hydrogen by various agencies to methylene blue are well known. Wurmser and Gelso[2] report a substance they call G′ which is developed in alkaline glucose solution protected from O_2 and which is capable of rapid oxidation in the cold by dehydrogenation in the presence of methylene blue or in the presence of molecular oxygen. They consider that probably it is a di-enolic form of glucose and that in the cell there exists an oxidation-reduction system similar to that of the glucose solution. It is possible that a similar factor is important in the reductions I have noted in mixtures containing sugars. Palit[3] and Palit and Dhar[4] have noted retardation of oxidation in the presence of fats or nitrogenous substances. This observation may be correlated with those to be described since successive reductions followed by delayed oxidation would result in a composite picture of more persistent reduction.

The solutions studied are listed on the opposite page. A control of each substance of the weight used in the mixture was run. In addition, three times the weight used in the mixture of two characteristic integrating substances (glycine and dextrose) were run as controls. The results observed for these corresponded closely with those noted for other controls. It is realized that contaminants of various metal salts were present. It is doubtful that these varied our results since they were uniformly present and many illustrative solutions were tested. All solutions were made up at both pH 5.6 and 8.0 (exception Nos. 13, 14, and 15 which were pH 5.0 and 8.0). They usually were run in triplicate and were rechecked by reautoclaving. With the exception of cysteine HCl solutions, where oxidation had precipitated the insoluble cysteine, results showed striking correspondence.

With the exception of cysteine solutions (Nos. 13, 14, and 15), because of rapid oxidation of the methylene blue, tubes were observed only with the eye. At both H-ion reactions the mixture of d-glutamic acid and succinic acid acted like the controls.

At pH 5.6 (Nos. 1 through 12) the controls showed little if any reduction of methylene blue. Mixtures with the exception of No. 5 showed complete reduction to the leucoform on removal from the autoclave followed by rapid complete oxidation in five to ten minutes.

At pH 8.0 (Nos. 1 through 12) controls and No. 5 showed none or very transitory reduction in no way to be confused with that of the mixtures. In the mixtures reduction was complete on removal from the autoclave to be followed by complete oxidation in one-half to two hours.

The factors which integrated with the sugars in the reduction apparently are not dependent on the amino group, since they are contained in succinic acid,

SOLUTIONS

(1) 1.0 gm. d-glutamic acid, 1.0 gm. dextrose
(2) 1.0 gm. d-glutamic acid, 1.0 gm. lactose
(3) 1.0 gm. d-glutamic acid, 1.0 gm. dextrin
(4) 1.0 gm. d-glutamic acid, 1.0 gm. sucrose
(5) 1.0 gm. d-glutamic acid, 0.6 gm. succinic acid
(6) 1.0 gm. d-glutamic acid, 0.6 gm. succinic acid, 1.0 gm. dextrose
(7) 1.0 gm. d-glutamic acid, 0.82 gm. succinic acid, 1.0 gm. dextrose
(8) 0.82 gm. succinic acid, 1.0 gm. dextrose
(9) 0.79 gm. maleic acid, 1.0 gm. dextrose
(10) 0.79 gm. fumaric acid, 1.0 gm. dextrose
(11) 0.52 gm. glycine, 1.0 gm. dextrose
(12) 0.62 gm. dl-alinine, 1.0 gm. dextrose
(13) 0.25 gm. cysteine HCl, 0.25 gm. d-glutamic acid
(14) 0.125 gm. cysteine HCl, 0.125 gm. d-glutamic acid
(15) 0.25 gm. cysteine HCl, 0.25 gm. d-glutamic acid, 0.25 gm. dextrose

maleic acid and fumaric acid. The factor peculiar to the sugars and apparently common to many sugars is present even in the "nonreducing sugar," sucrose, but to a lesser degree as indicated by persistence of reduction. Of the substances furnishing the other factors, glycine seems most active. The higher concentration of succinic acid seems to give a slightly more persistent reduction.

Various concentrations of cysteine HCl from 0.01563 gm. per 100 c.c. to 1.0 gm. per 100 c.c. of pH 5.0, 5.6 and 8.0 were studied. Results with 0.01563 per cent cysteine HCl corresponded to those of the mixtures discussed above. The reduction was more persistent in the more alkaline solution and less persistent in the acid solution. At a concentration of cysteine HCl intermediate between 0.125 and 0.25 per cent the relative length of time of persistence of reduction reversed, the acid solutions showing more persistent reduction. In acid solution (¼ per cent cysteine HCl) there appeared in the first few hours an initial oxidation after complete reduction by the autoclave. This oxidation was followed by a second reduction which persisted about eight-six hours.

In mixtures of d-glutamic acid and cysteine HCl, (Nos. 13 and 14) reduction was prolonged about twelve to twenty-four hours over control cysteine solutions at pH 5.0 but not 8.0. This is considered a second type of integrative action different in three ways from that previously discussed, (a) G′ factor cannot be postulated, (b) d-glutamic acid increases length of reduction in a system already powerful, and (c) the action is in the more acid mixture. In the later instance in which there has been change with H-ion concentration in the mixture showing integrative action sugar has not been used.

When a mixture of cysteine HCl, d-glutamic acid and dextrose (No. 15, pH 5.0) were autoclaved changes considered a third type of integrative action occurred. The solutions vacillated between 10 and 80 per cent reduction of methylene blue over the six days of observation. Similar mixtures at pH 8.0 showed no decided difference from control cysteine HCl solutions.

It is accepted that a state of vacillatory oxidation reduction occurs in living cells. It is hoped that a study of the third type of integrative action will give us valuable information that can be applied to cell processes.

The expression persistence of reduction is used in preference to inhibition of oxidation since it is assumed that forces at play are preventing more rapid accomplishment of the more stable oxidized state. The reduced state, because of greater unstableness, seems more emblematic of the phenomona of life. Certain substances and conditions aid in maintaining the reduced state. Since drying

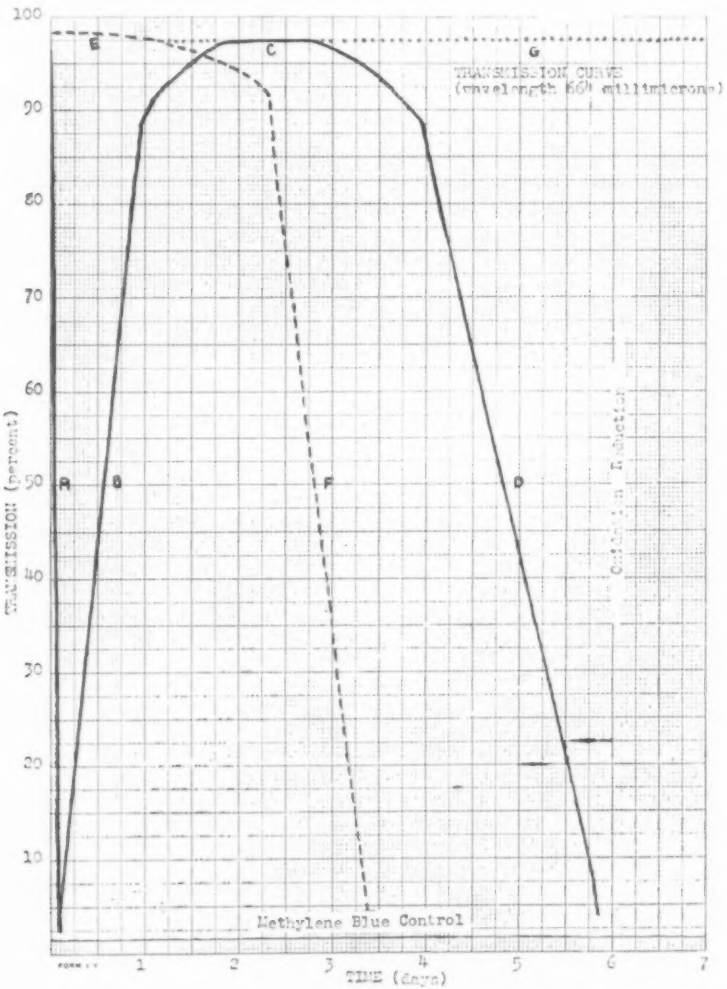

Chart 1.—The chart shows transmission curves for wave length 664 which has remained characteristic for 0.001 per cent methylene blue solution. The color variation was originally drawn by the Hardy spectrophotometer. On noting the percentages of transmission one notes the approximate percentage of reduced dye. The percentages must be corrected in accordance with the control methylene blue line in order to obtain more exact figures. It will be noted that neither complete oxidation nor complete reduction is attained.

Line *A* may be considered representative of what occurs when organic acids and sugars (pH 8.0) studied were autoclaved; there initially is noted 96 per cent reduced form which rapidly becomes oxidized so that only 1 per cent reduced form is charted.

Line *A* also appears in the more acid (pH 5.6) ¼ per cent cysteine solutions. It, however, again quickly reduces as seen in *BC* and is later oxidized (*D*). When glutamic acid is added to cysteine *D* may occur twelve to twenty-four hours later than in the control cysteine solution.

Curve *EF* is illustrative of 0.25 per cent cysteine at pH 8.0.

Line *ECG* is drawn to illustrate prolonged complete reduction (97 to 99 per cent) such as occurs in more acid (pH 5.0) concentrated (0.5 to 1 per cent) cysteine solutions.

The duplicate and triplicate curves studied were characteristic in type showing variations which seemed minor when one considers the variation probability.

and therefore concentration of reducing substances is one way of maintaining certain simple forms of life in a viable but inactive state, it may be presumed that when dilution of the cell's ingredients occurs (correct temperature aiding in establishing the ideal physicochemical state) sufficient oxidation takes place to establish the correct oxidation-reduction equilibrium for cellular growth.

The delay in oxidation noted when an amino acid was added to cysteine may be a phenomonon similar to that described by Barron and others[5] for ascorbic acid in which a heavy metal (copper) was the catalyst. It is probable, also, that in the systems studied, the rate of oxidation bears a relationship to the oxidation-reduction potential of the dye.[6]

Activation of change by heat may create spatial relationships which help determine reduction of the dye. It is evident that more study is necessary on type two and three integrative action. Since autoclaving was a basic factor in these studies and used as a point of departure in observation, changes in solutions which had not been autoclaved were not studied.

SUMMARY

The object of this paper is to report "integrative action," subsequent to autoclaving, expressed in persistence of reduction of methylene blue. Such action is divided into three types:

a. That of mixtures of organic acids and sugars which is more pronounced at pH 8.0 than pH 5.6.

b. That of mixtures of d-glutamic acid and cysteine HCl which is observable in solutions of pH 5.0 but not 8.0.

c. That of mixtures of d-glutamic acid, cysteine hydrochloride and glucose which shows a vacillatory state of partial reduction and partial oxidation in solutions of pH 5.0 but not 8.0.

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A STIRRING DEVICE FOR USE IN MICROTITRATIONS*

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THE use of an air current for stirring during microtitrations is an accepted procedure, but because of the lack of compressed air in some laboratories, or of proper reducing valves to control it, the method is not widely used. The device here illustrated is easily constructed and provides for adequate control of the air current.

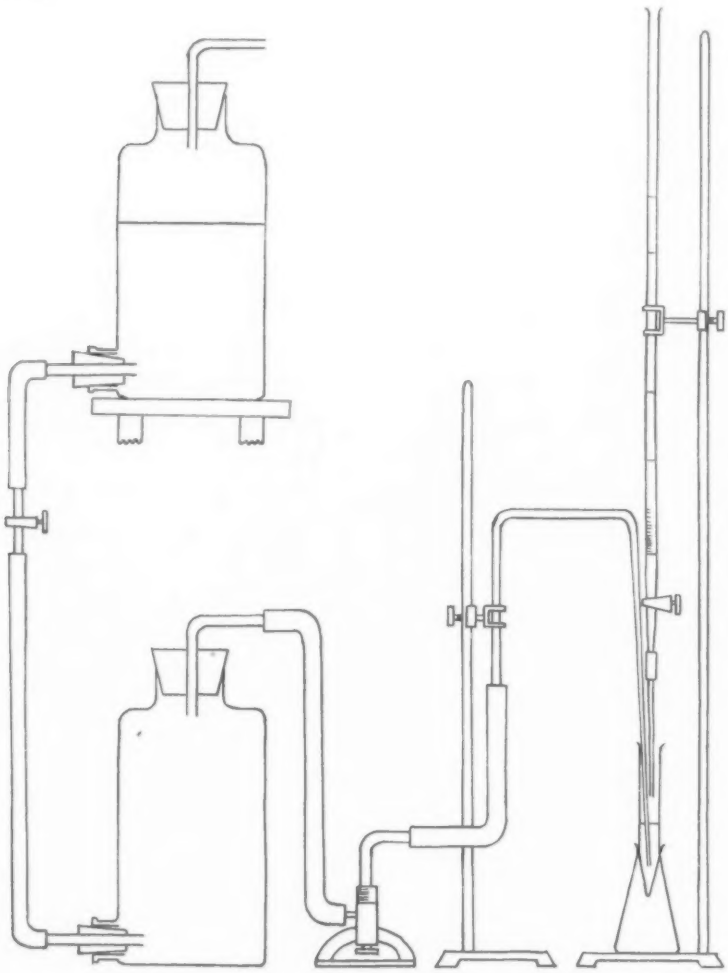

Fig. 1.

*From the Department of Biological Chemistry, Tufts College Medical School, Boston.
Received for publication, June 18, 1937.

The diagram is self-explanatory. Pressure is provided by water flowing from one aspirator bottle into another, thus displacing the air and forcing it first through the needle valve and then through the capillary tube which is immersed in the solution being titrated. When two 2-liter bottles are used, the supply of air will last for about an hour. After all the water has passed from one bottle into the other, the lower bottle may be disconnected and the positions of the two reversed. The needle valve is made from an old Tirrill burner by removing the barrel and soldering a short piece of copper tubing over the needle valve seat.

Since the volume of air used is so small, oxygen and carbon dioxide may be easily removed by connecting suitable absorption tubes in series, if either of these gases interfere with the titration.

A SIMPLIFIED SHAKING APPARATUS*

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IN SMALL laboratories, where expense of equipment is an item, a suitable shaking apparatus for serologic tests is frequently a factor of major importance.

The shaking apparatus described in this article was designed to fill the need for a simple and yet efficient and inexpensive means of shaking test tubes.

The apparatus consists of a complete motor vibrator, such as is used by many beauty parlors and barber shops for massage. The motor is clamped to a ring stand by an adjustable clamp, or may be bolted to a wooden base. The end of the vibrator, to which the rubber tip is attached, has a small threaded hole. By means of a screw passed through the bottom, a small mailing carton or baking soda can is attached firmly to the end of the vibrator and the junction soldered. Washers were used to reduce the strain on the thin bottom of the carton. When in use, the tubes are placed in the carton, the lid screwed on, and the motor started. This imparts a vigorous shaking motion, the speed of which may be controlled by means of a small rheostat.

An apparatus such as described above has been used by the writer for many months, and the results have been entirely satisfactory in every respect.

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*From the laboratories of West Texas Clinic and Hospital, Ranger.
Received for publication, June 21, 1937.

A SIMPLE COLORIMETRIC METHOD FOR DETERMINATION OF ALCOHOL CONCENTRATION IN URINE AND BLOOD*

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THERE is a definite need in legal and clinical medicine for a simple and accurate test for alcohol concentration in urine or in blood, a test which does not necessitate special distilling apparatus, and which may be applied anywhere, in places where elaborate laboratory facilities are not available.

Many alcohol tests have been described during the last few years, but they all have certain limitations, inasmuch as they lack simplicity, or require elaborate technique and apparatus.

Most of the methods are based on the oxidation of ethyl alcohol by potassium dichromate. The alcohol is either distilled from the specimen as in the method of Heise,[1] or absorbed by a current of purified air passing through the reagent (method of Bogen,[2]) or the alcohol is evaporated and absorbed by sulphuric acid in a hermetically closed flask (method of Widmark[3]).

Of the methods now available, the Widmark method is the most convenient, as it does not require distilling or aerating apparatus. It does require, however, a special S-shaped tube for absorption of the blood. This test calls for very small quantities of blood, but it is a titration method and necessitates skillful and elaborate technique. Abels[4] modified the Widmark method by transforming it into a colorimetric one. He also substituted filter paper for the S-shaped tube. As in the Heise and the Bogen methods, the partially decolorized potassium dichromate is compared with a series of standards, usually 15, each corresponding to a definite percentage of alcohol. As the standards are not stable, it is necessary each time to prepare a series of fresh standards, which makes these methods very tedious.

In the method herein described, the blood or urine specimen to be tested is evaporated in a tightly sealed flask, and the alcohol oxidized by potassium dichromate. The partially decolorized potassium dichromate is compared in a colorimeter with the potassium dichromate reagent. A dark blue filter is used to facilitate matching colors, as the partially decolorized potassium dichromate has a blue greenish tint, due to the presence of chromic sulphate.

As 1 c.c. of the potassium dichromate reagent corresponds to 1 mg. of alcohol, the percentage of alcohol in the specimen is determined from the difference in potassium dichromate contents between the reagent standard and the partially decolorized reagent. The chromic sulphate formed by oxidation of alcohol absorbs a certain amount of light in the colorimeter. This has been taken into consideration by diluting the standard and the unknown accordingly.

APPARATUS AND REAGENT REQUIRED

Fifty cubic centimeter Pyrex Erlenmeyer flask.

One rubber cork in which a monel or nichrome wire clip is inserted to keep the filter paper in place.

*From the Department of Pathology of the College of Medical Evangelists and the Laboratories of Los Angeles County Hospital, Los Angeles, Calif.

Received for publication, June 28, 1937.

Filter paper strips of medium thickness, 1½ by 2 inches.
Special blue filter.*
Accurate 2/10 c.c. pipette.

REAGENT

Solution of 0.4262 per cent of potassium dichromate in 50 per cent (by volume). Sulphuric acid (1 c.c. of this reagent corresponds to 1 mg. of alcohol.)

PROCEDURE

Roll one filter paper strip into a small cylinder and insert it into the clip attached to the cork. Introduce into the Erlenmeyer flask 1 c.c. of reagent. Absorb into the filter paper 2/10 c.c. of urine or blood to be examined.

Fig. 1.

The blood may be oxalated or freshly drawn from the ear lobe or the tip of the finger. Insert the cork immediately, but not tightly into the Erlenmeyer flask. Heat the neck of the flask from all sides over a Bunsen burner, and then press the cork very tightly into the neck of the flask. Place in a one liter beaker a small quantity of water, and into this put the flask. Adjust the volume of water in the beaker so that the flask does not float. Cover the beaker with a Petri dish or a watch glass. Boil the water in the beaker gently for fifteen minutes. Remove the flask from the beaker. Remove the cork gently from the flask, cool lower portion under tap, and add 4.3 c.c. of distilled water. Into a clean test tube introduce 2.5 c.c. of reagent and add to it 10 c.c. of distilled water. It will be seen from this that whereas 1 c.c. of reagent standard is diluted to 5 c.c., 1 c.c. of unknown is diluted to 5.4 c.c. Approximately 1/10 c.c. of the blood remains on the filter paper as unevaporated material.[4] The difference in the dilution is the necessary correction for absorption of light in the colorimeter by chromic sulphate. Compare in any microcolorimeter, using the blue filter and setting the unknown at 10. Calculate from the following formula:

$$(1 - \frac{R}{10}) \times 500 = \text{mg. Alcohol per 100 c.c.}$$

R = Reading of the reagent-standard.

For example: R is 7.4; then $(1 - \frac{7.4}{10}) \times 500 = 130$ mg. alcohol per 100 c.c.

*Lantern blue glass filter No. 554, manufactured by MacGregor Instrument Co. of Needham, Mass.

When the concentration of alcohol in blood or urine is high (above 250 mg. per cent), it is advisable to dilute the reagent standards by 2 or 4.

Comparative tests with normal blood and urine to which alcohol of a definite concentration was added: 100 mg. per 100 c.c. added to blood, and 200 mg. per 100 c.c. to urine. Alcohol in mg. per 100 c.c. was found.

Blood	102	104	101	103	102	104	102	102	101	106
Urine	196	201	198	203	200	195	204	198	202	190

In conclusion, I wish to express my thanks to Dr. Albert Chaney, Director of the Chemical Laboratory of Los Angeles County Hospital, for his kind cooperation during this work.

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THE HINTON TEST FOR SYPHILIS*

The Third Study of Its Clinical Value in 3,000 Patients

Lester Hollander, M.D., Clara R. Schlesinger, B.A., and Charles L. Schmitt, M.D., Pittsburgh, Pa.

WE HAVE again completed[1, 2] a comparative study in a group of 3,000 consecutive patients concerning the value of four laboratory procedures, the Wassermann (lipoid antigen), the Wassermann (cholesterinized antigen), the Kahn, and the Hinton. This report summarizes our findings.

The tables designated with Roman numerals refer to the first study of 3,000 patients; the tables designated with Roman numerals and the letter *A* refer to our second study of 3,000 patients, while the tables designated with Roman numerals and the letter *B* indicate our findings in the latest group of 3,000 consecutive patients.

Table I

General Summary

	I	IA	IB
Total number of individuals tested	3,000	3,000	3,000
Number of positives to one or more tests	655	622	427
Number of doubtful to one or more tests	16	11	10
Number of negatives to all tests	2,329	2,367	2,563

Table II

Comparison of the Methods Used

	II		IIA		IIB	
	POSITIVE	ANTICOMP.	POSITIVE	ANTICOMP.	POSITIVE	ANTICOMP.
Wassermann Lipoid	317	6	322	1	284	4
Wassermann Cholesterol	362	6	378	1	340	4
Kahn	400	0	505	0	359	0
Hinton	643	0	582	0	399	0

*From the Pittsburgh Skin and Cancer Foundation, Pittsburgh.
Received for publication, July 13, 1937.

TABLE III

ANALYSIS OF THE POSITIVE REACTIONS

WASSERMANN LIPOID	WASSERMANN CHOLESTEROL	KAHN	HINTON	III	IIIA	IIIB
+	+	+	+	297	316	279
+	+	−	+	15	0	2
+	+	+	−	1	0	0
+	+	−	−	1	3	3
+	−	−	−	1	1	0
+	−	+	+	1	5	0
−	+	+	+	33	48	42
−	+	+	−	1	0	3
−	+	−	+	6	3	4
−	+	−	−	5	4	4
−	−	+	+	61	126	32
Anticomp.	Anticomp.	+	+	3	0	4
−	−	+	−	3	7	3
−	−	−	+	227	109	51

TABLE IV

ANALYSIS OF THE HINTON ONLY POSITIVE PATIENTS

HISTORY	PHYSICAL FINDINGS	PREVIOUS ANTISYPHILITIC TREATMENT	IV	IVA	IVB
+	+	+	137	86	19
+	+	−	16	5	11
+	−	−	12	2	3
−	+	+	2	2	2
−	+	−	7	2	3
−	−	+	11	1	4
−	−	−	42	11	9

TABLE V

ANALYSIS OF THE UNCONFIRMED HINTONS

	V	VA	VB
Number	42	11	9
Spinal fluid Wassermann positive	23	1	2
Remaining unconfirmed	19	10	7

TABLE VI

ANALYSIS OF NEGATIVE HINTONS—POSITIVE TO OTHER TESTS

WASS. LIP.	WASS. CHOL.	KAHN	HISTORY	PHYSICAL	PREVIOUS TREATMENT	VI	VIA	VIB
+	+	+	+	−	+	1	0	0
+	+	−	+	−	+	0	1	1
+	+	−	−	−	−	1	2	2
+	−	−	−	+	−	1	0	0
+	−	−	−	−	−	0	1	0
−	+	+	+	−	+	1	0	0
−	+	−	+	−	+	3	2	1
−	+	+	−	−	+	1	0	2
−	+	−	−	−	−	2	2	3
−	−	+	+	−	+	0	3	0
−	−	+	−	+	−	0	2	0
−	−	+	−	−	−	2	2	4
					Total	12	15	13
			Considered unconfirmed			4	5	9
			Hinton failed in			8	10	4

COMPARATIVE VALUE OF THE HINTON TEST

In the present or third group of 3,000 patients, we found 427 serologically positive individuals. Of these, 399 gave a positive Hinton reaction. The test giving the closest number of positives was again the Kahn with 359. Fifty-one patients were negative to all other serological tests except the Hinton. Of these, 7 cases remained unconfirmed and are classified as false positives. All the other tests combined produced 9 unconfirmed cases.

The Hinton reaction failed in 4 cases which were positive to one or more of the other tests.

SUMMARY OF THE THREE STUDIES

In 9,000 patients, 1,704 patients gave a positive reaction to one or more of the four laboratory procedures used; 37 gave a doubtful result, and 7,259 were entirely negative.

Of the 1,704 positive cases the Wassermann lipoid was positive in 923 instances, the Wassermann cholesterol in 1,080 instances, the Kahn in 1,264 instances, and the Hinton in 1,624 instances.

Of the 1,704 serologically positive cases, 54 remained unconfirmed (being positive to one type of serological test only). In 9,000 consecutive cases, 18+ per cent gave positive evidence of having demonstrable syphilis.

CONCLUSIONS

1. On further study the sensitivity of the Hinton test in the serological examination for syphilis is still greater than any of the other tests.

2. Reliance cannot be placed on one serological test alone.

3. Thus far all of our studies substantiate our original premise that all available tests should be utilized in the establishment of the diagnosis of syphilis in order to give the best possible serological service in this disease.

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THE USE OF PARAFFIN RINGS FOR RAPID BLOOD TYPING*

CLARA M. BECTON, M.S., M.T., TULSA, OKLA.

LANDSTEINER[1] and Coca[2] and others have emphasized the importance of determining the agglutinins of human bloods as well as the agglutinogens when typing blood for transfusion. In order to determine the agglutinogen-agglutinin distribution in bloods, it is necessary to test the unknown cells with Groups A, B, and O serums and the unknown serum with A and B cells.

A simple method for determining the presence or absence of agglutinogens and agglutinins in blood is the use of paraffin rings in the same manner as for the Kline Precipitation Test on a 2 by 3 inch glass slide. Fifteen paraffin rings, 3/8 inch inside diameter, are placed on a 2 by 3 inch glass slide, 5 rows long by 3 rows wide; three unknown bloods can be tested at a time with this setup.

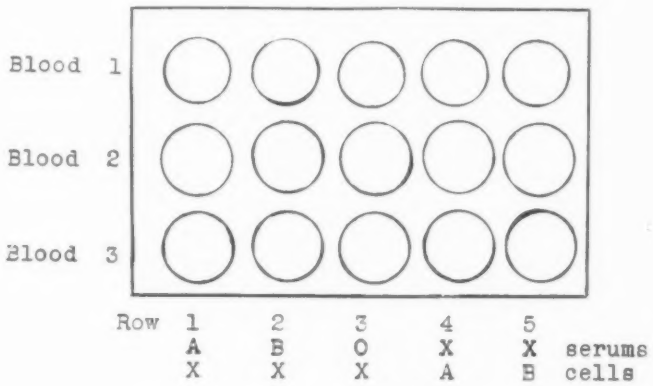

Chart 1.—Diagram for paraffin rings.

Light cell suspensions are set up in the following manner:

Row 1.	A serum	plus	X cells
Row 2.	B serum	plus	X cells
Row 3.	O serum	plus	X cells
Row 4.	X serum	plus	A cells
Row 5.	X serum	plus	B cells

The slide is rotated for three minutes and examined with the microscope for agglutination.

The four Landsteiner groups give the following reactions with this method:

GROUP	A SERUM	B SERUM	O SERUM	A CELLS X SERUM	B CELLS X SERUM
O*	-	-	-	+	+
A	-	+	+	-	+
B	+	-	+	+	-
AB	+	+	+	-	-

*-signifies lack of agglutination. +signifies agglutination.

*Aided by the Mark Finston Research Fund.
From the Department of Pathology, St. John's Hospital, Tulsa.
Received for publication, July 17, 1937.

The examination of blood for agglutinins is a very valuable check on typing. In case the A and/or B test serums are faulty, the use of Group O serum and A and B cells will indicate typing irregularities in most instances. Moreover, this method shows up defective and/or atypical blood groups in that there is a double check for the agglutinogens and agglutinins of the unknown blood.

The A and B cells must be fresh or preserved in a medium which prevents diffusion of agglutinogen. Unwashed cells suspended in their own serum and kept at icebox temperatures retain high agglutinogen content for a week at a time. The typing serums are standardized according to Weiner.[3]

This method has proved very rapid in our hands, thus saving much time when large numbers of bloods are to be typed. It is not recommended for compatibility tests. Evaporation must be avoided in the cross-matching of bloods, and only closed chamber or test tube methods are recommended for the actual compatibility test.

SUMMARY

1. A rapid method for blood typing, determining both the agglutinogens and agglutinins, has been described.

2. Paraffin rings are quickly prepared on and removed from glass slides, whereas cover slips require tedious handling if they are employed with slides in a procedure.

3. This method has the advantage over porcelain plates in that each agglutination setup can be examined with a microscope, thus eliminating the possibility of overlooking latent or weak agglutination.

I am indebted to Dr. I. A. Nelson for many valuable suggestions.

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BLOOD GROUPING FOR MAJOR BLOOD GROUPS WITH PLASMA AND OXALATED BLOOD CELLS*

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IN a discussion on the various uses of oxalated blood, Dr. I. A. Nelson, Director of Laboratories at St. John's Hospital, called my attention to the fact that there is no basic reason why potassium oxalate would alter the agglutinogens and agglutinins of human blood and that it should be possible to type as correctly with plasma and oxalated cells as with cells and serum.

In this series of experiments, cross-matching was not carried out, the object being to compare blood grouping with cells and serum and oxalated plasma and cells. The typing sera and plasma were obtained from the same individuals, and standardized according to Weiner.[1]

Five hundred bloods were typed in duplicate with serum and plasma. Precipitation of fibrin in the oxalated plasmas was avoided by eliminating the suspension of cells in saline and/or citrate, the oxalated cells being suspended in their respective plasma. Identical reactions were obtained in this series:

	A SERUM	A PLASMA	B SERUM	B PLASMA	FINAL GROUPING
12 Bloods	+	+	+	+	AB
42 Bloods	+	+	0	0	B
182 Bloods	0	0	+	+	A
264 Bloods	0	0	0	0	0

SUMMARY

Failure to agglutinate, or false agglutination did not occur in 500 bloods typed with plasma and oxalated erythrocytes.

CONCLUSIONS

1. Oxalated blood cells typed with plasma give the correct blood types.
2. Typing with plasma and oxalated cells saves laboratory time, especially if venous blood is used for routine blood counts, etc.

REFERENCE

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*From the Department of Pathology, St. John's Hospital, Tulsa.
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METHOD FOR CLEARING COAGULATED SERUM-BLOCKED BERKEFELD FILTERS

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DURING the course of some serologic procedure, some human serum was filtered through a small Berkefeld. Inadvertently the candle was placed at once into some boiling water for sterilization without taking the precaution of "washing" the serum out of the Berkefeld with water several times.

On attempting to use the candle on a later occasion, it was found that no pressure or suction effect could be obtained. The albumen had precipitated into the interstices of the filter and had blocked it almost completely.

A solution of egg albumen was tried (since precipitated proteins are supposed to redissolve in an excess), but the result was poor. Before trying any other solution through the Berkefeld, a solution of egg albumen was boiled and flocculated out in a test tube and various reagents added to see the effect. The following all increased the flocculation: xylol, acetone, ether, hydrogen peroxide, and ammonia. At last Clorox (a 5 per cent solution of sodium hypochlorite) was used. This cleared the egg albumen precipitate immediately.

By using strong continuous suction and full strength Clorox in the filter, the Berkefeld began to function fairly well in about five minutes, and in about fifteen minutes was completely cleared. It was washed through with plain water several times, boiled, and retested. It was then as good as new.

This is a method for clearing Berkefeld filters which have accidentally been blocked by coagulated protein material.

To test the further efficacy and usefulness of the filter the following test was performed: An actively growing seven-day broth culture of a hemolytic streptococcus was used. A minute quantity was transferred to a fresh sterile broth culture tube. The rest (about 7 c.c.), consisting of a very turbid fluid, was filtered through the Berkefeld. The filtrate was absolutely clear. A large amount of the filtrate was transferred to sterile broth for culture. All the cultures were incubated for several days. There was an active growth in the reseeded tube, but no growth in the culture of the filtrate. Hence, by the physical clearing of the filtered material and the sterility of the filtrate, the continued efficacy of the cleansed Berkefeld was demonstrated.

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THE DETERMINATION OF ALCOHOL IN BLOOD AND OTHER BODY FLUIDS*

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NUMEROUS methods have appeared during the past few years for the determination of alcohol in blood and the various body fluids. Most of the European methods have been modifications of the Widmark method[1] in which the distillation of alcohol into the standard dichromate-sulfuric acid mixture is carried out in a 50 c.c. glass stoppered Erlenmeyer flask. The bottom of the glass stopper carries a 0.5 c.c. cup attached by means of a glass rod. One-tenth cubic centimeter of the sample to be analyzed is placed in this cup. On standing two to twelve hours, depending on the temperature, all volatile material such as water, alcohol, etc., passes over into the dichromate-sulfuric acid mixture. To determine the amount of dichromate which did not react with alcohol, potassium iodide is added and the freed iodine titrated with thiosulphate. The dichromate destroyed by the alcohol indicates the amount of alcohol present in the sample.

It appears that Widmark's type of distillation has been used but little in America. The common practices used to isolate the alcohol from the blood or fluid are: some form of aeration, to add a protein precipitant and distill, or to prepare some type of filtrate and distill. It appears that although a waiting period is not required by the above procedures there is a greater chance for error and more work is involved.

Harger[2] presented an alcohol method in which he titrated the excess dichromate with ferrous sulphate and methyl orange in a 62 per cent by weight sulfuric acid solution. Methyl orange is destroyed by dichromate but as soon as an excess is added the solution turns pink, indicating the end point. It appeared that this method of titration would have some advantages if used with the Widmark distillation.

APPARATUS

A special 50 c.c. glass stoppered Erlenmeyer flask. The glass stopper carries a small cup (Fig. 1).

A 0.1 or 0.2 c.c. pipette graduated between two marks which, to give the desired accuracy, should be about six inches apart.

A 5 c.c. volumetric pipette.

A 5 c.c. Mohr pipette attached by a rubber tube to a stopcock and held by a buret clamp serves as an excellent buret for the titration. The tip may be drawn out until drops of 0.02 c.c. are delivered. The buret is filled by applying suction through a rubber tube attached to the stopcock.

*From the Laboratory of Physiological Chemistry, University of Minnesota.
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REAGENTS

Standard dichromate solution: Dissolve 0.4258 gm. of C.P. potassium dichromate in water and make up to one liter. One cubic centimeter of this solution is equivalent to 0.1 mg. of ethyl alcohol.[2]

Concentrated sulfuric acid C.P.

Sulfuric acid, 50 per cent by volume. Add 1 volume of concentrated sulfuric acid to one volume of water.

Methyl orange, 0.1 per cent solution. Dissolve 0.1 gm. of methyl orange in 25 c.c. of 0.1 N sodium hydroxide and make up to 100 c.c.

Ferrous sulphate, 20 per cent solution. Dissolve 5 gm. of $Fe\ SO_4 . 7H_2O$ in 15 c.c. of water. Add 3 c.c. of concentrated sulfuric acid and make up to 25 c.c. Keep in stoppered flask.

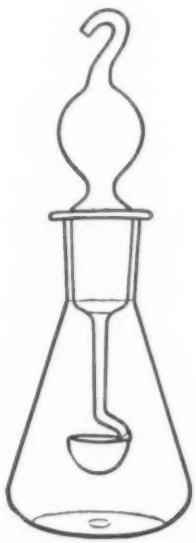

Fig. 1.

Red reducing fluid: To 35 c.c. of the 50 per cent sulfuric acid, add 15 c.c. of the 0.1 per cent methyl orange solution and 1 c.c. of the 20 per cent ferrous sulphate solution. Mix well and cool to room temperature. The red reducing fluid keeps only three or four days, but the solutions from which it is prepared are stable for several months.

METHOD

Place 5 c.c. of the standard dichromate solution in the special Erlenmeyer flask and add 5 c.c. of concentrated sulphuric acid. Place 0.1 or 0.2 c.c. of the blood or fluid to be analyzed into the small glass cup attached to the stopper and insert it into the flask. Allow the flask to stand for two hours in an oven at 70° C. or twelve hours (over night) at room temperature. Remove the stopper and cup, which contains the dried blood or fluid, and titrate the contents of the flask with the red reducing fluid. The yellow solution changes to a pink color at the end point. A blank determination must be made simultaneously. Its titration will require about 2.5 c.c. of the red reducing fluid.

Calculation: Since 5 c.c. of dichromatic solution are equivalent to 0.5 mg. of ethyl alcohol, twice the amount of red reducing fluid required to titrate the blank is equivalent to 1 mg. of alcohol. If the blank titration expressed in cubic centimeters is represented as B and that of the alcohol determination as A then

$\frac{B - A}{2B}$ = mg. of alcohol present in the sample. If a 0.1 c.c. sample has been used, this formula also expresses the per cent alcohol present in the blood or fluid. When a 0.2 c.c. sample is used, the value must be divided by two.

DISCUSSION

The principle involved in the Widmark distillation is the same as when sulfuric acid is used in a desiccator, except that the alcohol is destroyed by the dichromate. The blood or fluid is entirely dried in two hours at 70° C. Flotow and Nauenburg[3] have pointed out that allowing the flask to stand twelve hours, or over night at room temperature is just as effective, and the author has found this procedure to be somewhat preferable.

The glassware must be freed of all dust or grease by cleaning with chromic-sulfuric acid mixture and thorough rinsing. The distilled water must be of good quality. The Mohr pipette with a stopcock at the top is recommended in place of a buret for the red reducing fluid, because it can be filled by suction and the 50 per cent sulfuric acid does not come in contact with the stopcock. If the buret clamp is placed on the rubber connection between the pipette and stopcock it allows freedom of movement so that one can shake the flask while titrating.

When known amounts of alcohol are added to blood, the recovery is usually within 2 per cent of the amount added.

The blood may be taken direct from the finger tip or collected with oxalate either from the finger tip or vena puncture. Sterilization of the skin must be with 1:5000 mercuric chloride instead of alcohol.

Blood or urine kept in a refrigerator for twenty-four hours gives the same value as when the sample was taken, however after three or four days variations were found.

Blood from individuals who had received no alcohol gave values of 0.003 to 0.005 per cent.

The rule that one gram of alcohol by mouth per kg. of body weight will cause the blood to contain one gram of alcohol per liter (0.1 per cent), one to two and one-half hours later, was found to hold for individuals who were not habitual drinkers and had not eaten for several hours.

The alcohol content of urine and saliva is usually somewhat greater than that of the blood for some time after taking alcohol, but an equilibrium is reached after two to three hours.

The reaction time of individuals with different concentrations of blood alcohol was studied. The apparatus used had pedals representing the clutch, brake and accelerator of an automobile in which the time for manipulating the

various movements, after seeing a light flash, were timed with a Cenco impulse counter measuring to $\frac{1}{120}$ of a second. Three individuals on which several tests were made with the above apparatus when varying concentrations of alcohol were present in the blood gave the following average results.

0.05 per cent alcohol in the blood slowed the reaction time 7 per cent.
0.08 per cent alcohol in the blood slowed the reaction time 12 per cent.
0.10 per cent alcohol in the blood slowed the reaction time 19 per cent.

Miles[4] found that lid reflex, eye reaction time and eye movement velocity are slowed up by alcohol. The term "channel driving" has been used to express one's inability to note things approaching from the side when driving under the influence of liquor. In these experiments this phenomenon was noted in that the subjects were less disturbed by outside influences and were more attentive to the light signals after receiving alcohol than during the control determination.

With 0.1 per cent alcohol in the blood the subjects were able to walk a straight line and talk intelligently although they were somewhat talkative.

Brandt[5] states that most motorcycle accidents occur when the blood alcohol is 0.1 to 0.15 per cent and most automobile accidents when it is 0.1 to 0.2 per cent. Apparently the majority of accidents occur when the reaction time for manipulating the controls of an automobile has been decreased more than 20 per cent.

SUMMARY

A simple method requiring but little special apparatus has been presented for the determination of alcohol in blood, urine and saliva.

A short discussion of its use and interpretation of the results has been given.

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5. Brandt, W.: Die Alkoholbestimmung im Blut, Chem. Zeitung **60**: 485, 1936.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TUBERCLE BACILLI, in the Gastric Contents, Stiehm, R. H. Am. J. M. Sc. **194**: 940, 1937.

The increasing use of the tuberculin test combined with roentgen ray examination of the infected lung may manifest many cases of asymptomatic pulmonary tuberculosis.

Laboratory procedures frequently make possible determinations of the status of the lesion before the roentgenogram shows a change.

Tubercle bacilli were found in the gastric contents in 15 of 21 (71.4 per cent) minimal cases of pulmonary tuberculosis in which the sputum was negative.

The absence of tubercle bacilli in the gastric contents suggests an inactive lesion, or if active, that the prognosis is good. Retrogression occurred in 5 of the 6 cases in this series without the institution of treatment other than increased hours of rest.

The examination of the gastric contents for tubercle bacilli frequently gives positive evidence (71 per cent in this series) that the minimal infiltration is pathologically active and in need of treatment. The procedure is of inestimable value in helping to determine, especially in the asymptomatic cases, how long active treatment should be continued. One of the criteria of a healed lesion is the repeated absence of tubercle bacilli in the gastric contents as indicated by guinea pig inoculation. Strict adherence to this principle would result in fewer recurrences in the many patients with apparent but not real cures.

ANEMIA, Acute Hemolytic (Lederer Type), Giordano, A. S., and Blum, L. L. Am. J. M. Sc. **194**: 311, 1937.

Three cases of acute hemolytic anemia (Lederer type) are reported and the literature reviewed.

The general picture of the disease is drawn and the differential diagnosis discussed.

Acute hemolytic anemia is characterized by an acute onset with fever, rapid development of a severe anemia with the appearance of immature cells, marked reticulocytosis and leucocytosis, and tendency to complete recovery. The dramatic response to a single blood transfusion appears to be very characteristic. The anemia is usually of the normochromic type, with marked tendency to macrocytosis.

It is suggested that acute hemolytic anemia is not a rare occurrence, and that many cases of obscure anemia reported in the literature as well as reports on recovery from acute leucemia actually belong to this group.

The clinical recognition of Lederer's anemia is of greatest practical importance since the untreated disease has a high mortality rate while the prognosis of the treated disease is absolutely favorable. Blood transfusion is the treatment of choice.

The reported occurrence of spontaneous autohemagglutination in course of acute hemolytic anemia is observed for the first time.

Some theories as to the action of blood transfusion are briefly presented.

The etiology of acute hemolytic anemia is at present unknown, but its infectious nature is very strongly suggested. A comparison with the acute hemolytic anemia of Oroya fever is made.

ANEMIA, Sickle-Cell, in the White Race, Haden, R. L., and Evans, F. D. Arch. Int. Med. 60: 133, 1937.

Two white patients with sickle-cell anemia are reported.

The patients are sisters, born of Sicilian parents.

There is no history or physical finding suggesting an admixture of Negro blood.

Both patients have had the spleen removed, with marked benefit.

In one case the spleen was removed fourteen years ago and in the other five years ago.

Both patients continue to have mild hemolytic anemia but are clinically well.

In sickle-cell anemia there seem to be two factors in the excessive hemolysis characteristic of the disease, viz., excessive filtration of abnormally shaped cells and excessive fragmentation or early death of the cells.

The results obtained in these cases suggest that splenectomy should be employed more often in sickle-cell anemia if the spleen is large and the patient is seen early in the disease. It is not a cure but only an aid in treatment, since anemia persists.

LEUKEMIA, Monocytic, Montgomery, H., and Watkins, C. H. Arch. Int. Med. 60: 52, 1937.

Distinction is made between monocytic leucemia of the Naegeli and that of the Schilling type on the basis of the hemocytologic pictures. Either type may be of primary autochthonous cutaneous origin. The type of cutaneous manifestations of either condition may be specific or nonspecific and may vary from discrete necrotic nodules or purpuric lesions to generalize exfoliative dermatitis. A distinctive histopathologic picture of monocytic leucemia of the Schilling type may be observed on examination of the skin, corresponding to the hemocytologic picture. Occasionally, myelogenous leucemia may terminate as monocytic leucemia of the Naegeli type, or, as in Case 4, lymphatic leucemia may result in monocytic leucemia (Schilling) and still later again present the blood pictures of both conditions. Monocytic leucemia of the Schilling type (leucemic reticulo-endotheliosis) may assume an acute or chronic form, and even aleucemic reticulosis may be encountered.

It is important, therefore, to correlate the cutaneous as well as the general clinical, pathologic, and hemocytologic observations in a given case in order to arrive at a correct diagnosis. A prolonged period of observation may be necessary before it is possible to ascertain the type of lymphoblastoma that will eventuate.

DIARRHEA, Epidemic, in the New-Born, Rice, J. L., Best, W. H., Frant, S., and Abramson, H. J. A. M. A. 109: 475, 1937.

This report is a preliminary analysis of outbreaks of highly fatal diarrhea that have occurred among newborn babies in the nurseries of eleven lying-in institutions of New York City from July, 1934, to December, 1936. Among 3,672 liveborn babies, 505 cases of the disease occurred, a morbidity rate of 14 per cent. Of the sick infants 234 died, a mortality rate of 6.4 per cent. The case fatality rate was 46 per cent. Extensive bacteriologic investigations failed to reveal the inciting agent in the disorder. Autopsy did not shed any definite light on the pathogenesis of the disease.

The clinical syndrome was characterized by signs of acute intestinal toxicosis, probably of primary origin, and was accompanied by the symptom train of frequent watery yellow stools, abdominal distention, severe dehydration, drowsiness and acidosis, rapid weight loss, little temperature reaction, marked toxicity and prostration. Vomiting occurred occasionally; blood, pus, or mucus in the stools was infrequent. The main complications were terminal otitis media and bronchopneumonia.

From observations of the various outbreaks, and from a careful search of the literature, it is the authors' present impression that they are dealing with a well-defined clinical entity affecting infants of the newborn period of life, deserving consideration apart from other diarrheal disturbances of infancy. They feel that the condition is more widespread than a survey of the literature would lead one to believe.

TETANUS BACILLI, Isolation of, from Street Dust, Gilles, E. C. J. A. M. A. 109: 484, 1937.

This investigation was undertaken to determine the presence of tetanus spores in street dust. The method employed was to cultivate the material, isolate tetanus-like organisms, study their biochemical reactions and, finally, demonstrate the ability of the strains isolated to yield a spasm-producing toxin which could be neutralized by tetanus antitoxin.

As a preliminary to the isolation of pure cultures, it was necessary to grow the mixed material in special mediums to stimulate the growth of any tetanus organisms present and then to eliminate the vegetative forms of bacteria by heating the material at 80° C. for twenty minutes. Since, however, the spores of sporulating aerobes and facultative anerobes were present, the use of selective dyes in the medium was found necessary to eliminate them. The dye employed was crystal violet, which was found to be entirely satisfactory. In the separation of the tetanus organism from other spore-bearing anerobes, the method originally employed by Fildes was used. This procedure makes possible the isolation of tetanus organisms from a mixture of anerobes.

The biochemical reactions of the strains of *Clostridium tetani* isolated were studied, and their toxin-producing ability was demonstrated by the inoculation of animals. From the sixty-three samples of street dust obtained from various streets within a limited area of Baltimore and examined in this investigation, eleven strains of tetanus bacilli were isolated; that is, 17.4 per cent of the samples revealed the presence of the organism. Of these, nine strains, or 14.2 per cent of the samples, yielded a spasm-producing toxin neutralized by tetanus antitoxin, whereas two strains isolated from 3.17 per cent of the samples did not yield any detectable toxin.

The results obtained in this investigation do not permit of comparison with any other work of a similar nature, since apart from the early observations of Nicolaier and Bossano, no reports have been found in the literature of the isolation of tetanus organisms from street dust, and the present investigation appears to be the first successful isolation of tetanus bacilli from the street dust of a large modern city of the United States.

The positive results are definite and conclusive proof that *Clostridium tetani* is widely distributed in street dust even at the present day and establish the absolute necessity for the use of prophylactic injection of tetanus antitoxin in all cases of street accidents, accompanied by laceration or abrasion of the cutaneous surface. The fear of anaphylaxis can no longer justify the failure to employ protective serum in every case of street accident in which there are open wounds, since street dust, contrary to the views of many, has been shown to be a potential source of danger.

FUNGI, A Specific Chemical Medium for Pathogenic, Southworth, W. H. Arch. Dermat. & Syph. 36: 302, 1937.

The preparation of this medium, which may prove of interest to mycologists, is as follows:

Water	1,100.0 c.c.
Dextrose	40.0 gm.
Magnesium sulfate	0.5 gm.
Potassium dihydrogen phosphate	3.0 gm.
Dipotassium hydrogen phosphate	0.5 gm.
Sodium chloride	1.0 gm.
Agar	15.0 gm.
Urea	10.0 gm.

Dissolve the constituents, except the urea, in 1,000 c.c. of water. Put 250 c.c. of solution into each of four small-mouthed bottles. Sterilize the bottles in the autoclave at 15 pounds' (6.8 kg.) pressure for twenty minutes. Dissolve the urea in 100 c.c. of water, and

filter the solution through a Berkefeld filter. Add 25 c.c. of sterile solution of urea to each of the four bottles after they have been sterilized and cooled to about 55° C. Place in test tubes quantities of from 10 to 12 c.c. of the medium before the agar begins to solidify.

A mixture prepared according to these directions has a pH between 5.6 and 5.8

MARROW PUNCTURE, Sternal, in Infants and in Children, Kato, K. Am. J. Dis. Child. 54: 209, 1937.

A differential cell count of nucleated elements seen in bone marrow smears made with aspirated material from infants and children reveals certain characteristics which differentiate their picture from that of the adult marrow. These differences are (1) the relatively high percentage of erythrogenic and lymphoid elements in the marrow of young subjects, and (2) the relatively low percentage of myeloid elements due to a lack of more mature granulocytes. This reversal of the percentage in erythrogenic and myelogenic elements explains the low myeloid-erythroid ratio of 1:2 for infants and young children, older subjects normally having a value of 3:4.

The fact that the diagnosis was made possible by the sternal puncture method in a case of acute myelogenous leucemia and in a case of Niemann-Pick's lipoidosis illustrates the specific value of the method as a diagnostic procedure.

TUBERCULIN, Skin Sensitivity in Chronic Tuberculosis in the Course of Hospital Treatment, Hayes, M. G., Pastor, J. R., Gaetan, L. R., Cory, R. A. S., and Long, E. R. Am. J. M. Sc. **194**: 220, 1937.

Fluctuations in the intensity of the tuberculin skin reaction were studied by monthly tests in 116 ward patients with chronic pulmonary tuberculosis. Twenty patients remained under observation approximately one year and 67 for more than four months. To ensure uniformity, standardized tuberculin (Purified Protein Derivative) was used throughout the experiment. Two doses, viz., 0.00002 mg. (the standard first dose) and 0.000002 mg. (one-tenth of the standard first dose) were employed on each patient. In most cases one or the other of these detected the threshold of reaction.

The general level of sensitiveness to tuberculin was low. Strong reactions were never seen. Of the 20 patients remaining under observation approximately a year, not one averaged more than a 2-plus reaction to the stronger of the 2 doses. Eight of the 116 patients were negative to both doses for the whole course of their study. To the stronger dose 27 per cent of the entire group were negative, and 72 per cent to the weaker dose, two-thirds of the time.

Of the 67 patients injected at 5 or more monthly intervals, more than half maintained approximately the same level of sensitiveness, as did 12 out of 20 followed for approximately a year. The fluctuations in the remainder were of inconstant character, but certain consistent trends were observed. While, as is already well known, patients with unfavorable course tended to lose their tuberculin sensitiveness as the end approached, a definite continuing depression of sensitivity was observed also in patients with chronic tuberculosis of long duration, but somewhat favorable course. On the other hand, transient or prolonged increase in sensitiveness occurred in numerous patients with clinical improvement.

Observation was directed particularly toward the effect of special therapeutic procedures employed, and also toward such events as pulmonary spread of the disease, and particularly pleural effusion. Pneumothorax per se had no noticeable effect. In the small number of cases of thoracoplasty observed, a drop in sensitivity followed the operation. In the majority of cases pleural effusion appeared to depress skin reactivity.

No correlation could be established between tuberculin skin sensitivity and the patient's temperature or with seasonal influences.

TUBERCULOSIS, Agglutinins for Typhoid Group Bacilli in, Damon, S. R. Am. J. Hyg. 26: 40, 1937.

Serums from 143 cases of tuberculosis having negative histories of previous enteric infection or T.A.B. inoculation have been analyzed for agglutinins for the H and O antigens of *B. typhosus*, *B. paratyphosus B*, and *B. paratyphosus A*.

Eighty-seven (60.8 per cent) of these serums had no demonstrable content of agglutinins for any of these antigens in dilutions of 1:20 or over.

Fifty-six (38.4 per cent) of these serums contained agglutinins for one or more of these antigens in dilutions of 1:20 or over.

The titers for the H antigens frequently exceeded that generally taken as diagnostic (1:100) in persons having no history of infection or vaccination.

The titers for the O antigens never exceeded that taken as diagnostic (1:100) in individuals never having had enteric infection or prophylactic vaccination and seldom exceeded 1:20.

These results suggest that in the serologic diagnosis of enteric infections in persons suffering from tuberculosis, unreliable results may be obtained in a large percentage of cases when the examination is made by the classical Widal test. They also indicate that these misleading results may be obviated by the use of a qualitative receptor analysis.

However, following this procedure it is to be noted that the H antigen cannot be employed with serums from tuberculous individuals even though the serums come from patients having negative histories of previous enteric infection or prophylactic vaccination. Ordinarily in serum from such persons, agglutination of the H antigens in diagnostic titer is taken as indicating not only enteric infection but also the species of infecting microorganisms. In serum from tuberculous patients the frequent appearance of nonspecific reactions invalidates any such conclusion, and we are forced to fall back on the reactions with the O antigens. Therefore, we may perhaps conclude that as agglutinins for the O antigens in sufficiently high titer to be significant have never been encountered in our experience in serums from tuberculous patients, we may use this reaction to detect definite enteric infection without indication of the infecting species of organism. However, here, as in all such agglutination tests, the demonstration of a rising titer of O antibodies, in the patient's serum, on repeated tests, carries relatively more weight than the absolute titer in any single examination.

TUBERCLE BACILLI, in Gastric Washings of Infants and Children. Am. J. Dis. Child. 54: 48, 1937.

The finding of tubercle bacilli in the gastric contents, as determined by the production of tuberculosis in a guinea pig inoculated with the sediment obtained from the gastric washings, is indicative of the presence of an active tuberculous focus. This lesion is usually either in the lung or in a hilar or tracheobronchial lymph node which has ulcerated into the bronchus.

Tubercle bacilli were demonstrated in the gastric washings in 7 (8.14 per cent) of a series of 86 infants and children with positive cutaneous reactions to tuberculin in whom no recognizable evidence of tuberculosis could be demonstrated on physical or roentgenographic examination.

Examination of the gastric washings for tubercle bacilli by the inoculation of a guinea pig with the sediment should be done for all infants and children with positive cutaneous reactions to tuberculin, even if no evidence of tuberculosis has been found on physical and roentgenographic examination. The presence of tubercle bacilli in 8.14 per cent of the children in our series in the absence of physical and roentgenographic findings indicates the necessity of such a procedure, since the diagnosis of active tuberculosis could not have been made in these cases without the positive evidence obtained by examination of the gastric contents. These patients would have remained an unrecognized source of tuberculous infection to uninfected children with negative cutaneous reactions to tuberculin.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor, Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

Practical Talks on Kidney Disease*

DISEASE of the kidney is a diagnostic and therapeutic problem with which the physician is frequently confronted. It is unfortunate, therefore, that this subject is—and with some justice—regarded as both difficult and confused.

The title of this book is no misnomer, for it is, indeed, an eminently practical discussion of kidney disease in general and in particular. Dr. Weiss evidences not only a thorough understanding of his subject, clearly fortified by ample clinical experiences but he possesses also the ability to impart information in a clear-cut, simple, and readily understandable manner.

The book is not intended as a monograph or textbook but rather as a practical presentation of the subject. In this it is exceptionally successful.

It is divided into six main sections in which are discussed: The Mechanism of Kidney Function; Disturbances of Renal Function; Signs and Symptoms of Kidney Disease; Glomerulonephritis; Nephrosis; Pregnancy and Kidney Disease; and Nephrosclerosis.

While the book will prove of value to the student and physician alike, in its preparation Dr. Weiss has performed a service of inestimable value to the practitioner to whom this little volume should prove invaluable.

Genital Abnormalities, Hermaphroditism, and Related Adrenal Diseases†

STATED to be the most comprehensive treatise yet published upon these subjects, this is in many respects a remarkable book.

Dr. Young, whose reputation in the field of genitourinary surgery is far-flung, has had the unique opportunity of studying fifty-five cases which are reported in detail, and the surgeon will be particularly interested in the various surgical procedures which have been utilized and devised for their treatment. These are described and illustrated in detail. The anatomist, physiologist, pathologist, and physician alike will find Dr. Young's book invaluable as a reference volume.

The book is an outstanding example of a thorough, comprehensive, and authoritative study, and an excellent illustration of the printer's art at its best.

The numerous original drawings by William P. Didusch and the many photographs, together with the text, make this an epochal volume which for some time to come will be the outstanding reference in this field.

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*Practical Talks on Kidney Disease. By Edward Weiss, M.D., Professor of Clinical Medicine, Temple University School of Medicine. Cloth, 176 pages, 10 figures, $3.00. Charles C. Thomas, Springfield, Ill.

†Genital Abnormalities, Hermaphroditism, and Related Adrenal Diseases. By Hugh Hampton Young, The Brady Urological Institute, Baltimore. Cloth, 680 pages, 534 illustrations, $10.00, Williams and Wilkins Company, Baltimore, Md.